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THE MICROBIOLOGY OF LONG CHAIN FATTY ACID DEGRADATION IN THE NORTH SASKATCHEWAN RIVER

by

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A THESIS

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled

THE MICROBIOLOGY OF LONG CHAIN FATTY ACID DEGRADATION IN THE NORTH SASKATCHEAN RIVER

submitted by RICHARD NIGEL COLEMAN, R.T. (C.S.L.T), B.Sc. in partial fulfillment for the degree of Master of Science.

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Date Oct. 8, 1975

ABSTRACT

A direct plate count method has been developed for the enumeration of long chain fatty acid utilizing bacteria present in fresh water. It was concluded from observing the colonies growing on the solid medium (TYA+FA) that the medium was acting in a selective manner.

In later experiments the medium(TYA+FA) was used in conjunction with a non-selective medium(TYA) to attempt to assess the North Saskatchewan river's capacity to degrade long chain fatty acids. North Saskatchewan river water was sampled from a site on the river upstream from Edmonton(156 St.) and from a site downstream from Edmonton(Vinca bridge). To evaluate the effect of the city of Edmonton upon the North Saskatchewan river, the Edmonton domestic sewage plant effluent was sampled at the time of the river sampling. During the summer period of 1974 the source waters of the North Saskatchewan river were also sampled to determine any changes that occur in the river during its passage from the source to Edmonton. In a parallel study, the occurrence of salmonellae in the North Saskatchewan river and the Edmonton domestic sewage effluent was determined using the Moore swab technique (Moore, 1948). In addition to the microbiological

experiments, various physical and chemical parameters were measured.

Several conclusions were made regarding the river's capacity to degrade long chain fatty acids. Long chain fatty acid degradation appears to be secondary to other bacterial metabolic capabilities. There was no stastically significant correlation between the numbers of long chain fatty acid utilizing bacteria(FAU) and the long fatty acid levels within the river. The North Saskatchewan river sediment contained twice as much long chain fatty acid per volume at the Vinca bridge sample site as compared to the sediment at 156 St. Sediment at these two sites always appeared black and had a distinct odor of hydrogen sulphide. Long chain fatty acid non-utilizing bacteria(FANU), which are usually greater than 90% of the total bacterial count, showed a tendency toward being pigmented. Analysis of both the sediment and the water at the source site revealed the long chain fatty acid levels to be very low as compared to the levels found in other sediment. In addition, all bacterial counts were also low at the source site. Contrasting the other river sites, the source yielded only aerobic bacteria whereas the other sites contained both aerobic and facultative anaerobic microorganisms. The presence of Salmonella sp. in the North Saskatchewan river water was investigated and were found

on 1 of 8 occasions and at both local river sites. The Edmonton domestic sewage effluent was also monitored and salmonellae were found on 3 of 8 occasions. The source, however, yielded no salmonella.

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LIST OF ABBREVIATIONS

g .....gram

mg .....milligram

ug .....microgram

ng .....nanogram

pg .....picogram

l .....litre

ml .....millilitre

mM .....millimolar

SD .....Standard Deviation

dpm .....disintergrations per minute

rpm .....revolutions per minute

v/v .....volume per volume

w/v .....weight per volume

12:0 .....dodecanoic acid

14:0 .....tetradecanoic acid

16:0 .....hexadecanoic acid

16:1 .....cis-9-hexadecenoic acid

18:0 .....octadecanoic acid

18:1 .....cis-9-octadecenoic acid

18:2 .....cis,cis-9,12-octdecadienoic acid

18:3 .....cis,cis,cis-9,12,15-octadecatrienoic acid

r .....correlation coefficient

FAU .....fatty acid utilizer

FANU .....fatty acid non-utilizer

B-oxidation .Beta oxidation

INTRODUCTION

Long chain fatty acids occur in nature (Viswanathan *et al.*, 1962; Given *et al.*, 1973) to such an extent that the accumulation of this class of compounds could present a major biological degradation problem. Man has tended to concentrate large quantities of long chain fatty acids in relatively small areas in the form of industrial and domestic sewage treatment plant effluents.

In spite of the fact that natural aquatic systems such as rivers or streams are used as receiving waters for such wastes, methods have not been developed to ascertain the presence of long chain fatty acid utilizing microorganisms. To date no record of work in this area has appeared in the literature. The metabolism of closely related compounds such as triacylglycerols has been studied. These components, when hydrolyzed, yield glycerol and the so-called R-groups which are primarily long chain fatty acids. The release of these organic acids with the concomitant drop in pH is the basis of the pH indicator colour change detection method for triacylglycerol hydrolysis (Sierra, 1957), whereas other methods usually rely on the clearing of the insoluble triacylglycerols surrounding colonies. This system, then, determines those microorganisms having the ability to hydrolyze

triacylglycercls but yields no information concerning the degradation of long chain fatty acids.

In sanitary engineering technology the 'greases' are those materials that when acidified to pH 1 are extractable into hexane or other non-polar solvents. This procedure would tend to hydrolyze most triacylglycercls to glycerol and long chain fatty acids. Many workers have shown that up to 70% of the total greases are saturated and unsaturated long chain fatty acids(Viswanathan et al., 1962;Walter, 1961). In natural systems such as rivers and sewage systems there is an abundance of lipolytic microorganisms, therefore the supply cf free long chain fatty acids should depend on the supply of triacylglycerols.

In the design of a method for the detection of microbial long chain fatty acid utilization it must be considered that the oxidative enzymes required for the breakdown of these compounds have been found to be associated with or bound to the bacterial cell membrane(Weeks et al., 1969). Therefore the classical enumeration procedures based on the formation of clear zones surrounding colonies cannot be used.

Reports in the literature have shown that microorganisms present in sewage influents, sewage effluents and other situations high in concentrations of

organic material are predominantly of the non-pigmented variety such as coliforms, pseudomonads and aeromonads(Jones, 1970;Coleman *et al.*, 1974). The non-pigmented microorganisms are introduced to the receiving waters along with sewage effluent and long chain fatty acids. Two possibilities arise as to the fate of the fatty acids, the long chain fatty acids may be utilized either by the introduced bacterial flora or by the indigenous river populations. The bacteria introduced via the sewage effluent could also be pathogenic to users of the receiving waters. Rivers and other natural water bodies contain a high proportion of pigment-producing species, greater than 50% of the total population in some cases(Coleman *et al.*, 1974). Many workers have ascribed the term microbial scavengers to these pigmented microorganisms, that is, they can exist in very dilute organic medium(Jones, 1970).

The problem of detection of pathogenic bacteria present in sewage effluents and subsequent receiving waters has plagued Public Health officials for years. Many workers, particularily in the U.S.A., have attempted to correlate the presence of 'fecal coliforms', 97% of which are *Escherichia coli* , with the probable presence of enteric pathogens, in particular, those of the genus *Salmonella* (Geldreich, 1966). This infers that the survival physiology of 'fecal coliforms'

is similar to that of Salmonella sp. (McCoy, 1964). Other public health workers, especially in the United Kingdom, have attempted recovery methods to demonstrate the presence of salmonella directly.

The objectives of this research and thesis are as follows:

1. To develop a direct and simple plating method for the enumeration of long chain fatty acid utilizing microorganisms (FAU).
2. To evaluate a natural environmental situation using the developed method with other measurable parameters that may have an effect upon the numbers of FAU.
3. To attempt to determine whether fatty acid utilization is a simple substrate-microorganism relationship or whether any other(s) measurable parameter(s) affect activity.
4. Concomitant with the problem of evaluating an environmental situation, a direct method was used to survey for the presence of Salmonella sp.

LITERATURE REVIEW

I. Rivers as microbial systems.

Rivers have long been used as the receiving environment or system for man's wastes because of one unique fact, that is, whatever is discharged into the river is carried away from the depositor. As rivers, in general, can degrade all or at least a major portion of the wastes that are deposited therein, they may be considered as moderately active microbiological systems. Wuhrmann(1971) in his chapter on the self purification process in rivers attributes this ability to the biochemical activities of the indigenous microbial populations present. He makes a further observation that self purification is highly dependent upon the absolute bacterial biomass, that is, the number of bacteria that are in intimate contact with the flowing water. In simplistic terms, the degradation of many insoluble substrates requires the intimate contact of the substrate with the competent microorganism(Wuhrmann, 1971).

The fact that rivers are metabolically active, with this activity mainly attributed to microorganisms, leads to the question as to where, within the river, the activity lies, that is, what is(are) the habitat(s) of the microorganisms present in the river? Wuhrmann(1971) considered that the river's microbial populations are

essentially allochthonous in origin or that they are introduced to the river. Soil, sewage and other terrestrial leached materials are the main contributors to this group of bacteria. The point that a river is essentially a dilute medium, possibly similar to a dilute soil solution, is well taken in that all tributary water had their origin on land. The river partially compensates for this dilution effect by the fact that the water flow delivers to the sessile or fixed cell, fresh substrates.

A river consists of two phases, the water and the sediment. In the water phase there are essentially two microbial populations, the free living cells and the epiphytes or those organisms attached to larger objects such as plants, animals and detritus. The sediment contains the epipsammic flora or those living in and on the substratum. On a volume basis, the sediment contains the largest proportion of the river's microbial population. Jannasch (1956) reported that 99.6% of the total river microbial population of the river Nile are present in the sediment. A similar distribution of the microbial population was found in another river system and under widely differing climatological conditions (Coleman et al., 1974). There appears to be no difference in the microbial counts in either the sediment or the water if they are expressed on a per dry

weight basis. This implies a soil-like situation in the aqueous phase, in that the microbial cells are possibly associated with particulate material. The conclusion as to the numbers of bacteria is somewhat simplistic since the bacterial counting method involved only aerobic incubation. The sediment is deficient in oxygen and will contain undetected anaerobes that are in a metabolically active condition. The water, as it is actively aerated, would probably contain few if any anaerobes. Therefore the sediment, if evaluated by aerobic plate count, will contain more bacteria than the plate count reveals.

Bacteria are of an ubiquitous nature and are therefore varied in their nutritional requirements and metabolic activities. It would appear valid to say that any particular environment selects for the competent autochthonous or naturally present population and against any incompetent allochthonous microorganisms. Although Wuhrmann(1971) states that most river-born microorganisms are allochthonous in origin, their continued preservation within the river system would indicate an allochthonous to autochthonous shift. This being the case and rivers being dilute solutions of many different nutrients, the microorganisms present would possibly grow better on laboratory growth media of lower organic concentrations. Jones(1970) specified that

increasing the organic content of a medium, in particular yeast extract, tends to lower the total numbers of the natural bacteria obtained from rivers. Jones(1970) also stated that the method described in Standard Methods for the Examination of Water and Wastewater using plate count agar or phytone glucose yeast extract agar incubated for 48 hrs was inadequate. This method gave significantly lower counts than a method using a more dilute medium incubated for a longer time. Coleman et al. (1974) reported that the use of a dilute medium gave higher counts than did plate count agar in a microbial survey of the North Saskatchewan river.

Various compounds have been incorporated into agar and the clearing of the compound from the medium was followed to observe bacterial degradative activity. Classically, clearing zones surrounding the competent colony indicated an extracellular degradative enzyme system. Triacylglycerols and lipids have been incorporated into solid media as a means of observing their degradation(Blaise and Armstrong, 1973). Generally, the lipid substrates have been natural oils such as cotton seed oil(Blaise and Armstrong, 1973) or Tween 20, 40 or 80(Sierra 1957). All of these assays evaluate the ability of the growing colonies to hydrolyze the ester linkage of the insoluble or

suspended lipid to give clear zones around the colony.

II. Characteristics of microorganisms that are able to utilize lipids and long chain fatty acids.

The methods used for the detection and classification of lipolytic bacteria in natural environments are well documented in the literature. Blaise and Armstrong (1973) found that the major lipolytic bacterial groups in the Ottawa river were members of the genera Pseudomonas, Acinetobacter, Moraxella and Aeromonas. Druce and Thomas (1970) showed that 93% of the lipolytic bacteria isolated from an untreated water supply in Wales were Gram negative rods and were classified as members of the genera Acinetobacter and Pseudomonas. Soils and waters have also been shown to contain lipolytic bacteria most of which were of the genus Pseudomonas (Breuil and Gournot, 1972).

The lipase system studied in vitro by Nantel and Proulx (1973) was dependent on the presence of calcium ion and an anionic detergent. The bacterial enzyme was most active at alkaline pH and polar mono- and diacylglycercls were more easily hydrolyzed than triacylglycercls. The latter ccmpounds required a higher concentration of anionic detergent to facilitate enzyme action.

Specific microbiological and biochemical studies using ^{14}C labelled substrates have shown that some bacteria and yeasts can take up and use long chain fatty acids. For example Penicillium roqueforti can oxidize long chain fatty acids to the corresponding methyl ketone with one less carbon atom(Lawrence and Hawke, 1968). It is also known that spores of P. roqueforti can also oxidize hexadecanoate into a homologous series of carbonyl compounds(Dartey and Kinsella, 1973). In E. coli K12 the long chain fatty acid oxidation system present is similar to the B-oxidation system found in mammalian tissues. This system was found to be induced by the presence of long chain fatty acids greater in chain length than C14. These microorganisms can obtain energy and assimilable carbon from the metabolism of long chain fatty acids(Weeks et al., 1969).

Long chain fatty acids are oxidized to acetyl CoA units after having been activated by an acyl CoA synthetase. The acetyl CoA is then fed into the tricarboxylic acid cycle where it is presumably further oxidized to carbon dioxide and water(Weeks et al.,1969). It has been suggested, but with no supportive evidence, that carnitine transports long chain fatty acids across the bacterial membrane as it does in the mitrochondrion(Overrath et al., 1969). Since the time of this postulation evidence has been accumulating to

indicate that the transport of long chain fatty acids into the cell is by a process called vectorial acylation. The enzyme responsible for this process is Acyl CoA synthetase and is found to be membrane associated with two activities. The first is that of activation of the long chain fatty acid and the second is the transport into the cell of the cleaved acetyl group(Klein et al., 1971).

III. Long chain fatty acids as antibacterial agents.

Long chain fatty acids, in particular those greater in chain length than C12, have been shown by some workers to have antibacterial activity, particularly against Gram positive bacteria. For instance the MIC(Minimal Inhibitory Concentration) of cis-9-octadecenoic acid for Bacillus megaterium was found to be 0.05 mM or 13.4 mg/l and that of hexadecanoic acid was 0.3 mM or 78 mg/l. None of the Gram negative microorganisms tested including Pseudomonas aeruginosa were inhibited by concentrations up to 1.0 mM or 260 mg/l. The inhibitory effect can be reversed by the addition of either calcium, magnesium or cholesterol(Galbraith et al., 1971). However, there is no information relating to the inhibitory effect of combinations of long chain fatty acids, especially in environmental situations. Antibacterial activity and

the uptake of long chain fatty acids have been found to increase with increasing acidity and carbon chain length. In these experiments the long chain fatty acid uptake was cell associated rather than a result of intracellular transport of the label. That is, the label remained with the original acid but was physically bound to the cell surface rather than vectorially acylated, transported and metabolized to acetate(Galbraith and Miller, 1973b). These authors also found that the bactericidal activity was adversely affected by alkaline earth metals such as Al^{3+}, Ba^{2+} and Fe^{3+}. Galbraith and Miller(1973a) attributed the sensitivity of bacterial cells to a membrane association of the long chain fatty acid followed by some membrane disruption. The increased sensitivity of Gram positive cells was due to the high internal osmotic pressure, which resulted in the rupture of the cell(Hugo, 1967).

IV. Incidence of long chain fatty acids.

1. Eucaryotic sources.

In general homeothermic animals and plants contain mixtures of saturated and unsaturated long chain fatty acids. Usually these acids are esterified to glycerol to give triacylglycerols which in most organsims are storage fats. White rat livers, for instance, contain mostly triunsaturated triacylglycerols with the acyl

component being for the most part cis-9-octadecenoic acid, followed by cis,cis-6,9-octadecadienoic acid and hexadecanoic acid. Poikilothermic animals living at low temperatures generally have a higher concentration of unsaturated fatty acids which tend to lower the melting point of the storage fat. This allows the cell components to remain liquid at lower temperature (Leninger, 1970; Goldfine, 1972). Ruminant storage fat contains mostly saturated long chain fatty acids, presumably any dietary unsaturated long chain fatty acids would be biohydrogenated by rumen bacteria (Dawson and Kemp, 1970).

2. Procaryotic sources.

Long chain fatty acids present in procaryotes are similar in most respects to those found in eucaryotes. Degradation of long chain fatty acids is carried out by similar enzyme sequences, the exception being that the first enzyme in the B-oxidative sequence, acyl CoA synthetase, is cell membrane bound in the procaryot and mitochondrial associated in the eucaryot. It is generally considered that bacterial lipid systems do not contain di- and triunsaturated octadecanoic acids. The monounsaturated octadecanoic acid present is often not cis-9-octadecanoic acid or oleic acid but rather cis-11-octadecenoic acid or vaccenic acid (Leninger, 1970; Goldfine, 1972). Other unique long chain fatty

acids have been detected in bacteria such as B-hydroxy carboxylic acids and cyclopropane containing long chain fatty acids. A cyclopropane long chain fatty acid is one containing a double bond between the the 9 and 10 carbon across which a methylene group has been added thus forming a cyclopropane group. The precursor long chain fatty acid for cyclopropane long chain fatty acid formation is presumably cis,cis,cis-9,11,15-octadecatriencic acid with subsequent hydrogenation of the remaining unsaturated bond after the addition of the methylene group(Goldfine, 1972).

It would appear that some definite differences and some similarities exists between procaryotic and plant eucaryotic long chain fatty acid synthesizing or transport systems. In terms of certain unsaturated long chain fatty acids, procaryotes have only cis-11-octadecenoic acid whereas eucaryotes contain cis-9-octadecenoic acid, cis,cis-9,11-octadecadienoic acid and cis,cis,cis-9,11,15-octadecatrienoic acid. Both procaryotes and plants however contain hexadecanoic acid, cis-9-hexadecenoic acid and octadecanoic acid thus giving some degree of similarity as well(Goldfine, 1972;Leninger, 1970).

3. Sewage effluent sources.

It has been estimated that as much as 12.5% of the organic material in sewage effluents are lipids and long

chain fatty acids, of which, two thirds are hexadecanoic, octadecanoic and cis-9-octadecenoic acids(Walter, 1961). Other workers have found that these 3 long chain fatty acids were present at total concentrations of 7.0, 0.61 and 34.5 mg/l, repectively, in anaerobic and aerobic treatment effluent and raw wastewater(Visvanathan et al., 1962). Hunter and Heukelekian(1965) have indicated the existance of 4 classes of long chain fatty acid containing lipid in sewage; free fatty acids, glyceride(acylglycerol), phospholipid and detergent fatty acids. These authors state that glyceride and free fatty acids constitute the major components of sewage lipid material. In both cases unsaturated long chain fatty acids predominate.

V. Long chain fatty acid detection

1. Extraction techniques.

Total lipid extraction techniques can be divided into 2 categories. The first is partitioning in a separatory funnel which is accomplished by shaking the sample with solvent followed by a standing period to allow separation into 2 phases. Alternately, heat and a reflux apparatus or a Sohxlet apparatus can be used to extract the lipid material (Radin, 1969;American Public Health Association, 1971). All systems use either a mixture of moderateley polar and nonpolar solvents or

nonpolar solvents alone.

2. Detection of long chain fatty acids.

a. Thin layer and paper chromatographic techniques.

The detection of free long chain fatty acids in simple systems usually involves solvent extraction followed by either thin layer or paper chromatography. These separations are done with ease in that resolution is rapid and there is little interference due to contaminating compounds. The long chain fatty acid components of a mixture of lipids can be separated from the mixture by thin layer and reverse phase chromatography. There is, however, no liquid chromatographic technique that will separate the individual long chain fatty acids. The detection of the long chain fatty acid areas on the developed chromatogram can be achieved by spraying with iodine or sulfuric acid (Radin, 1969) or protoporphyrin IX (Sulya and Smith, 1960).

b. Spectrophotometric methods.

After extraction of lipid material the total long chain fatty acids present in a sample can be estimated spectrophotometrically by reacting with Rhodamine B:uranyl acetate to produce a dye complex which absorbs light at a wavelength of 545 nm (Mackenzie et al., 1967). The dye has a high affinity for long chain fatty acids

and forms a stable complex with them. There are 2 limitations however, the dye complex is only active in nonpolar solvent and the analysis yields only the total long chain fatty acids present.

c. Gas liquid chromatographic techniques.

The most definitive methods for long chain fatty acid detection, resolution and estimation utilize the gas liquid chromatograph. Methyl esters of the long chain fatty acids are prepared and injected onto a column containing an inert support usually coated with diethylene glycol succinate(Farrington and Quinn, 1973). Workers have found that this method will detect the 3 major long chain fatty acids of sewage effluents, hexadecanoic, octadecanoic and cis-9-octadecenoic acids (Walter, 1961;Viswanathan et al., 1962).

Problems have occurred in relating the peak area from the detector response to the relative concentrations of the individual fatty acid species. It has been observed that there was a linear response between peak area and concentrations of individual fatty acids within a sample, therefore any one fatty acid methyl ester can be used as a standard for any other(s) (Ettre and Kabot, 1963). The range of fatty acids used by these authors was from octanoic acid to cis-9-octadecenoic acid.

d. Miscellaneous methods.

Methods have been published in the literature that will separate the lipid components and the long chain fatty acids present in a sample. A method utilizing column chromatography and a packing of Sephadex LH 20(Pharmacia Fine Chemicals) consisting of hydroxypropyl ether side chain linked to a dextran polymer, and an eluent of nonpolar solvents is reported to give seperation. This method is not however widely used because of its low recovery and poor resolution of the individual long chain fatty acids.

VI. Occurrence and enrichment of salmonellae in natural environments.

1. Occurrence.

Detection of members of the genus Salmonella in natural waters has and will be a problem, since only a small fraction of the human population are carriers. Other contributors are industrial sources such as abbatoirs, chicken and swine processing plants and contamination from other animal carriers such as the natural fauna(Geldreich, 1970). The problem then is one of finding and growing a microbial population that is low in numbers compared to the allochthonous population. Therefore a primary inoculation of river water onto a highly selective solid medium will generally yield

negative results. Moore(1949) initiated the early studies on the problem of retrieval and concentration of Salmonella sp. He devised a swab that was approximately 8cm square consisting of bandaging wrapped in a strong coarse gauze attached to a cord to facilitate sampling. Following sampling the swab was immediately placed into a selective medium. This method has been used with little modification by many workers with a high°C of success(Cherry et al., 1972;Spino, 1966; Dutka and Bell, 1973;Yoshe-Purer et al., 1971;Kampelmacher and Van Noorle Jansen, 1973;Ryan, 1972).

Attempts have been made to enumerate the numbers of Salmonella sp. present in aqueous environmental situations with varying°C of success. One such technique involved the filtering of large volumes of water onto cell retaining membranes and the subsequent inoculation of the membrane to selective medium. The Most Probable Number(MPN) technique was used where varying amounts of water were filtered and the filters placed in selective medium. This method has tended to produce results comparable to the Moore swab technique but with the reported advantage of being quantitative(Hendricks, 1971;Dutka and Bell, 1973). A distinct disadvantage inherent in this method is that very large volumes of water are required for filtration and that the portable filtration equipment is difficult

to move into inaccessible areas.

In water, the presence of Salmonella sp. is positively correlated with the numbers of fecal coliforms(Geldreich, 1966). This indirect method only infers the presence of Salmonella sp.(Geldreich, 1966, 1970;Van Donsel and Geldreich, 1971). Other workers, however, have concluded that there is no correlation between the numbers of fecal coliforms present and the numbers of Salmonella sp. present(Gallagher and Spino, 1968).

2. Enrichment.

In most cases of sub-clinical salmonellae infections, low numbers of bacteria are found in the feces of a carrier. Therefore introduction of such fecal material to a sewage treatment process only further dilutes the numbers of salmonellae present. The Moore swab sampling technique increases the chances of trapping microorganisms present in water and, in so doing, the problem of retrieval then becomes one of enrichment and selection. Selenite broth has been and is still used by many workers as an enrichment medium for Salmonella sp. from human and animal fecal samples(Martin, 1970;Ryan, 1972;Wilson and Miles, 1966), from sewage(McCoy, 1964;Grau and Smith, 1972), from both natural and polluted waters(Foliguet and Doncoeur, 1972;Cherry et al., 1972;Coleman et al., 1974) and from

river and stream sediments(Hendricks, 1971;Van Donsel and Geldreich, 1971) with high°C of success. Other enrichment media have been used such as tetrathionate broth (McCoy, 1962;Cherry et al., 1972) , selenite-cysteine broth(Cherry et al., 1972) and mannitol-selenite-cysteine broth(Grau and Smith, 1972).

The incubation temperature has a marked effect on the viability and subsequent recovery of Salmonella sp. from mixed populations. High temperatures have been shown to be lethal for non-salmonellae in selective medium. Spino(1966) has recommended that the selective broth be incubated at 41.5°C since he found that Salmonella sp. could be detected in water where the E. coli levels were found to be 220/100ml. These results have been confirmed by others(Van Donsel and Geldreich, 1971;Cherry et al., 1972;Dutka and Smith, 1973). Higher incubation temperatures such as 42°C(Yoshe-Purer et al., 1971) and 43°C(Grau and Smith, 1972;Kampelmacher and Van Noorle Jansen, 1973;Ryan, 1972) have been used for enrichment with good success.

3. Detection of salmonellae in enrichments.

The detection of Salmonella sp. in enrichment medium has been made on a large variety of solid media such as SS-agar, EMB agar(Wun et al., 1972)and brilliant green sulfa agar(Cheng et al., 1971), bismuth sulphite agar(McCoy, 1962). It appears that many of the methods

for *Salmonella sp.* isolation are equivalent in efficiency and that selection of a procedure depends to a large extent on personal preference (Wun *et al.*, 1972). Methods for the identification of the resulting colonies tends to follow the biochemical schema laid down by Edwards and Ewing (1972) which, as they recommend, is to be followed by serotyping.

MATERIALS AND METHODS.

A. SAMPLING PROCEDURES.

I. Sample sites.

The river sample sites were chosen so that the effect of Edmonton and its domestic sewage effluent upon the North Saskatchewan river could be evaluated. In addition, access to these sites was possible throughout the sample year. The information given in Table 1 describes these 3 sites. Normally the North Saskatchewan river at 156 St., a site upstream from Edmonton, is frozen for approximately 5 months of the year beginning, for example in 1973, on November 15 with final breakup occurring on the evening of April 21, 1974. Vinca bridge is a site downstream from Edmonton where a narrow central channel was continuously open throughout winter ice conditions. The ice at these sites was approximately 3 feet thick where samples were taken. Sampling dates were determined so that all events occurring over the year could be fully described by the parameters laid down in this study. The Edmonton domestic sewage effluent, 6 feet before entering into the North Saskatchewan river, showed little change over the sampling year (Table 1).

Samples from the source waters of the North Saskatchewan river were taken during the summer. In

TABLE 1

Description of Sample Sites and River Conditions on Sampling Dates

Sample Date	156 St.	Sewage Effluent	Vinca Bridge
	Site on Western outskirts of Edmonton. (No effect of the city on the river)	Effluent channel six feet before confluence with the river	Site 52 miles downstream from 156 St. and 39 miles downstream from sewage effluent site
6/9/73	River open, sampled in three feet of water, sixty feet instream, southbank	Sampled effluent channel;	River open, sampled in three feet of water, thirty feet in stream, southbank
14/11/73	Heavy ice flow, sample taken twenty five feet instream, three foot depth, southbank	As above; no ice	River open, sample taken at three foot depth, twenty feet instream, northbank
24/11/74	Total ice cover, sampled fifty feet in from south bank, nine inch auger	As above; no ice	Ice cover except narrow channel, sampled fifty feet in from north bank with nine inch auger
2/4/74	As above	As above; no ice	As above
22/4/74	River open, break up previous night, thirty feet in from southbank in three feet of water	As above	River open, sampled eight feet in from northbank in three feet of water
29/4/74	As above	As above	As above
21/5/74	As above	As above	As above
15/7/74	As above	As above	As above

order to compare the quality of the river at the source with that at 156 St. and Vinca bridge sites, water and sediment sample were taken during the summer of 1974.

The following plates are photographs of the sample sites used throughout this study. Plates 1, 2, 3 and 4 show the source, 156 St., domestic sewage effluent and Vinca bridge, respectively.

II. Sample collection.

a. Water samples.

Surface North Saskatchewan river water samples were taken at a point where the river's depth was approximately 3 feet. Sampling was performed in this manner because the depth of the river changes, thus, a surface sample would tend to standardize the procedure. The depth of 3 feet was found to be the optimal working depth for sampling when wading in the North Saskatchewan river which is a fast flowing river. Three, 4 litre samples of surface water were taken at the same time and from an identical point within the river. All water samples were collected by immersing the container in the river water with the opening facing upstream. To minimize bacterial growth, samples were stored at or near 1°C in an ice-water filled insulated container until bacterial analysis, approximately 4 hours later. City of Edmonton sewage effluent samples were obtained

PLATE I

A PHOTOGRAPH DEPICTING THE SAMPLE SITE
AT THE SOURCE OF THE NORTH SASKATCHEWAN RIVER

PLATE 2

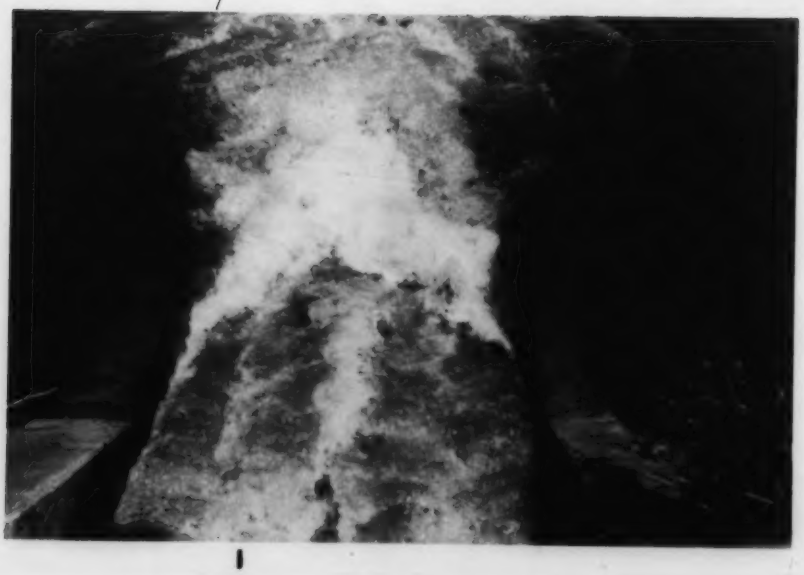
A PHOTOGRAPH DEPICTING THE SAMPLE SITE

AT 156 STREET ON THE NORTH SASKATCHEWAN RIVER

PLATE 3

PHOTOGRAPHS DEPICTING THE SAMPLE SITE AT

THE EDMONTON DOMESTIC SEWAGE EFFLUENT SITE



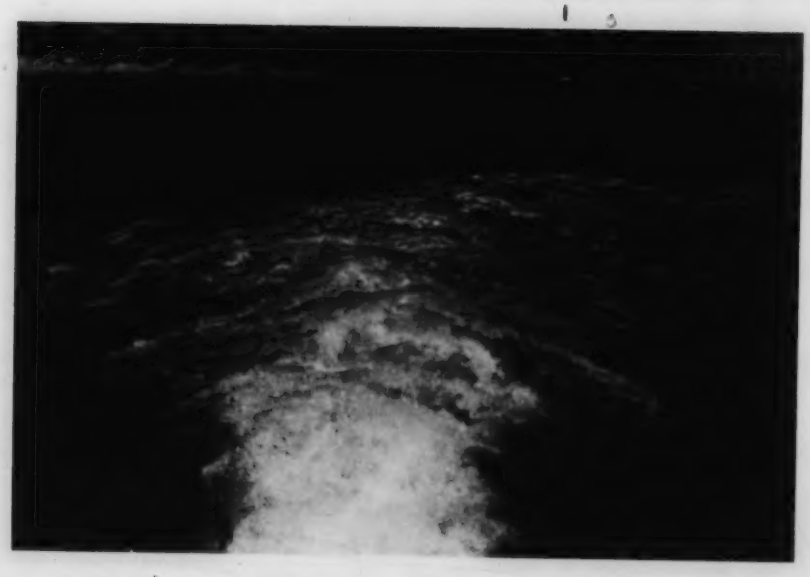


PLATE 4

A PHOTOGRAPH DEPICTING THE SAMPLE SITE AT
VINCA BRIDGE ON THE NORTH SASKATCHEWAN RIVER

from the final effluent channel about 6 feet before confluence with the North Saskatchewan river.

b. Sediment samples.

Sediment samples were obtained using a 4 foot section of 8 inch diameter poly vinyl chloride pipe, serrated on one end. On the other end 2 diametric holes had been cut to facilitate insertion of a bar. The assembly was lowered into the water where the depth was slightly less than 3 feet, rotated by means of the bar and pressed into the sediment to a depth of approximately 8 to 10 inches. The water overlying the sediment was removed with a pump. Samples were then taken with a scoop taking care to obtain a core of sediment which was not contaminated with seepage water. Samples were stored in an ice-water slurry until analysis. This method was chosen since the sediment in this river is heterogenous to the point where small commercial samplers such as the Eckman dredge would not close.

III. Sample handling.

All water samples were shaken on a reciprocal shaker at 278 reciprocations per min for 5 minutes. This was followed by a 5 minute sedimentation period to obtain uniform particle size in the aliquots taken for

analysis. Aliquots for dilution for microbiological analysis and for dry weight purposes were taken at the 8 cm depth. Subsequently 2 litres were poured from each sample site replicate into a common container and stored at -20°C for chemical analysis.

Sediment samples were handled in a similar manner with the exception that the shaking period was extended to 15 minute in order to remove attached material. In order to have some similarity of treatment between water and sediment a volume of 10 ml of mixed sediment was transferred to a dilution blank marked at 100 ml and filled with 90 ml of dilution buffer. This was then considered a 1/10 dilution of sediment.

IV. Physical analysis.

Sample dry weights were obtained by drying 3 sample replicates at 85°C, followed by dessication over annydrous calcium sulphate or phosphorous pentoxide until constant weight was achieved.

Temperature was taken *in situ* whereas pH was taken both *in situ* and upon arrival at the laboratory. There was little difference between the two pH measurements.

B. MICROBIOLOGICAL.

I. Sample dilution and plating for bacterial enumeration.

All water sample dilutions were made in cold 0.3 mM phosphate buffer, pH 7.2 (American Public Health Association, 1971). Sample aliquots were dispensed in 0.1 ml amounts to each of 5 plates of TYA for the total count and to TYA plus sodium octadecanoate(TYA+FA) for the long chain fatty acid utilizers. All agar surfaces were covered with the sample aliquot using the spread plate technique.

TYA or tryptone-yeast extract-acetate agar has the following composition and a final pH of 6.9-7.3 after sterilization.

Tryptone(Difco).....2.0g
Yeast extract(Difco)..2.5g
Sodium acetate.....0.2g
Agar(Difco).....15.0g
Water.....1 litre

All TYA plates were incubated at 25°C for 4 days(Coleman et al., 1974) at which time a differential count was performed yielding the total and the pigmented counts. Plates for the enumeration of the long chain fatty acid utilizers were incubated for 10-14 days at 25°C (100% relative humidity). A total count was then performed to yield the total long chain fatty acid

utilizing count. The method is described in a later section.

In order to detect long chain fatty acid non-utilizers, an incubated TYA plate from the total count analysis was replica plated to TYA+FA and TYA agar plates. The 2 plates were incubated at 25°C for 10 and 4 days, respectively. The position and characteristics of the colonies produced on the TYA plate were then compared to those produced on the TYA+FA plate. All of the colonies growing only on the TYA plate were considered as long chain fatty acid non-utilizing cultures(FANU).

II. Maintenance of cultures.

All long chain fatty acid utilizing isolates were picked and stored in maintenance culture consisting of nutrient agar(Difco) slants with deep butts. In many cases this procedure maintains a higher°C of viability than lyophilization(Antheunisse, 1972,1973). The butts were stabbed, the slants streaked, the tubes sealed with plastic caps and stored at 25°C.

III. Detection of long chain fatty acid utilization in liquid culture by a lipase producing P. aeruginosa ATCC 9027.

P. aeruginosa ATCC 9027 was grown at 25°C in tryptone-yeast extract-acetate broth (TYB) for 48 hours on a rotatory shaker at 300 rpm (eccentricity 1 in). Tryptone yeast extract acetate broth has the following composition and a final pH of 6.9-7.3 after sterilization.

Tryptone (Difco).....2.0g
Yeast extract (Difco)..2.5g
Sodium acetate.....0.2g
Water.....1 litre

The cells were harvested at 4°C by centrifugation at 10,000 rpm for 15 min. They were washed 3 times in cold 0.3 mM phosphate buffer pH 7.2 (American Public Health Asscciation, 1972) and finally resuspended to a density of one absorbance unit at 600nm (corresponding to approximately 2.6×10^8 cells per ml). Duplicate flasks containing either 0,50 or 362.5 ug/ml each of sodium hexadecanoate and sodium octadecanoate plus 250 ml of TYB were inoculated with 1 ml of the latter cell suspension and incubated under the following conditions. All flasks were shaken on a rotatory shaker at 300 rpm for 5 days at 25°C with daily removal of 5 ml samples. The samples were acidified to pH 1 with concentrated

hydrochloric acid and extracted by refluxing for 4 hours with 4 times the volume of chloroform-methanol(2:1). The lower chloroform layer was removed. The aqueous layer was washed 3 times with 20 ml volumes of the solvent, the lower chloroform layers removed and pooled. These chloroform layers were dried under nitrogen and the residue divided into 2 equal portions and assayed for lipid material and long chain fatty acids. The first assay involved the oxidation of the lipid material by dichromate(Saito and Sato, 1960). The second assay was for the analysis of long chain fatty acids using gas liquid chromatography.

The utilization of long chain fatty acids by P. aeruginosa ATCC 9027 was confirmed by following the metabolism of ^{14}C labelled long chain fatty acids in liquid culture. This technique involved additions of 200 mg per litre each of uniformly labelled(UL) ^{14}C sodium hexadecanoate and UL ^{14}C sodium octadecanoate(specific activity 12.5 DPM per mg; Amersham/Searle Corp., Arlington heights, Illinois, U.S.A.) to TYE at pH 8.0. This medium was inoculated with 1 ml of washed and resuspended P. aeruginosa cell preparation and incubated at 25°C. The inoculated medium was sparged with filter sterilized air and the effluent gases passed through a gas washing bottle(Fisher Scientific) containing a saturated

solution of barium hydroxide(Fisher reagent grade) to trap carbon dioxide. Radioactive barium carbonate was acidfied to ca pH 1 with concentrated hydrochloric acid. The ^{14}C carbon dioxide was flushed into phenethylamine counting fluid(Woeller,1961). The ^{14}C content was determined using an Isocap 300 Liquid Scintillation instrument(Nuclear Chicago) with subsequent correction for quenching. The efficiency of this trapping system was determined by acidification of a known weighed amount of sodium bicarbonate with concentrated hydrochloric acid. Any carbon dioxide released was trapped in the gas washing bottle containing saturated barium hydroxide. Precipitated barium carbonate was filtered, dried at 105°C and weighed. The loss of barium carbonate was 2.4% indicating that the gas trapping system was 97.6% efficient for trapping carbon dioxide.

IV. Detection of bacterial utilization of long chain fatty acids on solid medium.

In order to detect bacterial utilization of long chain fatty acids, a medium consisting of TYA plus fatty acid was used but with the pH ajusted to 8.3 with sodium hydroxide before sterilization. The basic pH was necessary to stabilize the added sodium octadecanoate as the salt. TYA agar plates containing 1% w/v sodium

octadecanoate were inoculated with P. aeruginosa ATCC 9027 and incubated at 25°C until 0.5 to 1.0 cm diameter colonies had developed, usually taking 10 to 14 days. Incubated plates were then sprayed with a 1:1 solution of Rhodamine B:uranyl acetate dissolved in various organic solvents. This reagent reacts with long chain fatty acids and is used in paper and thin layer chromatography(Mackenzie et al., 1967). The solvent in this reagent extracts the long chain fatty acid and gives a uniform color reaction across the plate which would not allow detection of long chain fatty acid utilization. The method of Sulya and Smith(1960) was tested which involved the use of an acidic solution of protoporphyrin IX dimethyl ester(Sigma chemical corp., Saint Louis, Missouri, USA). This reagent reacts with lipid and long chain fatty acid to produce a brilliant red chromagen when viewed under ultraviolet light. However, when applied to the medium used in this study there was no reaction.

It was observed that when a TYA+FA plate inoculated with P. aeruginosa ATCC 9027 was allowed to dry to a thin layer the colonies appeared almost transparent. After removing a colony a clear area was exposed in the agar directly under the preexisting colony. The surrounding medium remained translucent. This suggested that if a thin layer of medium was used there would be

little distance between the growing colony and the bottom of the plate. Thus, if utilization of the substrate occurred, the medium would clear directly under a utilizing colony. To prevent dessication of the medium, water saturated air was introduced into an incubator. An air system was designed such that air passed through an air stone submerged in water and was vented to the incubator chamber. To obtain the thin agar layer, it was found that 13 ml of medium if dispensed at a temperature of 80°C would cover the bottom of a petri plate. Following pouring and rapid cooling the plates were inverted and 'cured' at room temperature for 3 days. Such plates were inoculated by spreading 0.1 ml aliquots over the surface of the medium. These plates were then dried at room temperature for 3 hours and incubated in the 25°C incubator. Total counts of colonies growing on TYA+FA plates yielded the total FAU bacteria present. In each sample all colonies growing on the TYA+FA plate used for this count were picked and placed in maintenance culture. All plates were then bathed in a buffered salts solution (dipotasium hydrogen phosphate 7.0g, potassium dihydrogen phosphate 3.0g, ammonium phosphate 1.0g per litre) containing 1.0% Triton X-100 (Weeks et al., 1969) for 4 hours. The colonies were then removed with a glass spreader. Areas directly under the colonies showed clear zones indicating utilization of

the long chain fatty acid. This ability of such colonies to use long chain fatty acids was confirmed by the production of ^{14}C carbon dioxide from ^{14}C labelled long chain fatty acids.

V. Determination of the optimal concentration of sodium octadecanoate in solid medium for the detection of bacterial long chain fatty acid utilization.

Concentrations of sodium octadecanoate varying from 0.001% to 1.0% w/v were added to TYA(pH 8.3) prior to sterilization. The medium, after autoclaving, was held at approximately 80°C and dispensed as previously outlined.

A river water sample taken from Vinca bridge was diluted, incculated to TYA and TYA+FA plates and incubated as previously described. A total count and a count of the colonies clearing the TYA+FA were determined and compared to the total count on the TYA plate. Photographs were taken of one TYA+FA plate both before and after colony removal to reveal the difference in the typical characteristics of the plate due to bacterial action.

VI. Bacterial classification.

The characterization procedures used for each isolate were sufficient to classify them to the generic level. A characterization key similar to that used by Blaise and Armstrong (1973) and Westlake et al. (1974) was used with some slight modifications to conform to Bergeys Manual of Determinitive Bacteriology, 8th edition (Buchanan and Gibbons, 1974).

All 650 isolates were characterized by determining their Gram reaction, oxidative or fermentative utilization of glucose, oxidase test, flagella arrangement and colonial description. The Gram stain used was a modification of Hucker's method as described in the Manual of Clinical Microbiology (Blair et al., 1970). Oxidase activity was detected by the addition of a 1% solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (Eastman Kodak Co., Rochester, NY, USA) directly to the growing colony as outlined in the Manual of Clinical Microbiology (Blair et al., 1970). Glucose utilization was observed by the production of acid and gas in Board and Holding (1960) medium. In order to test for glucose utilization, a duplicate series of 5 ml deeps of the medium were stabbed with the isolate to be tested, one tube was incubated aerobically and the second anaerobically in a Gas-Pak jar with the appropiate gas generating

system (Baltimore Biological Co., Cockeysville, Maryland, USA). All cultures were incubated at 25°C for 5 days. Flagella patterns were determined by electron microscopy. Cells from a fresh exponentially growing TYB culture of the isolate was placed on a copper grid for flagella observation. The cells on the grid were then stained with 3% phosphotungstic acid and viewed under an electron microscope. The grid had a size of 400 mesh and was formvar (Ladd Research Industries Inc., Burlington, Vermont, USA) treated and carbonized. The electron microscope used was a Phillips model 200 (Phillips Electronics Industries Ltd., Holland). After observation of in excess of 100 isolates, flagella examination was discarded since the other characterization tests would permit differentiation in most cases to the genus level. The following insert was the key used to characterize the bacterial isolates.

COLOR	OXIDASE	GLUCOSE AERO	GLUCOSE ANAERO	FLAGELLA	GENUS OR FAMILY
Cream	+	A	-	Polar	Pseudomonas
Cream	-	AG	AG	Peritrich	Enterobacteriaceae
Cream	+	AG	AG	Polar	Aeromonas
Cream	+	-	-	Polar or Peritrich	Alcaligenes
Cream	+or-	Aor-	-	-	Acinetobacter
Yellow	+	Aor-	-	+or-Polar	Flavobacterium
Yellow	+	A	Aor-	-	Cytophaga

VII. Enrichment, isolation and identification of salmonellae.

A modification of the Moore swab technique was employed in order to detect the presence of Salmonella sp. in the environment. The swab consisted of 2 inch squares of cotton batting wrapped in a fine dacron gauze covering and stapled together. A 10 foot cord was attached through the center to allow suspension of the swab. The site was sampled by placing the swab in the current for usually 0.5 or 1 hour(4 hours was used in the case of the source sample site). After the specified exposure time the swab was removed and placed in 100 ml of filter sterilized selenite broth(Difco), returned to the laboratory(Hendricks, 1971) and incubated in a water bath at 43°C for 48 hours (Grau and Smith, 1972;Ryan, 1972;Kampelmacher and Van Noorle Jansen, 1973). Following incubation, 0.1 ml aliquots were streaked on 5 plates of fresh Wilson and Blair

bismuth sulphite(Baltimore Biological Co.) agar. The plates were incubated at 37°C for 48 hours(McCoy, 1962). Typical salmopellae colonies(dark brown) were picked and inoculated on to plate count agar, streaked on a urea agar slant, Simmons citrate agar, 10% lactose agar slants and stabbed into a Kliglers iron agar deeps(all Difco products). The tubes were incubated at 37°C and the reactions at 24 hours were compared to standard charts(Edwards and Ewing, 1972). All suspect isolates were tested against polyvalent Salmonella O antiserum to confirm the biochemical tests. The isolates were subsequently placed in serogroups and serotypes by the staff at the Provincial Laboratory of Public Health(Alberta).

C. CHEMICAL.

1. Extraction of lipid material.

Chloroform-methanol, 2:1, is reported to be the best general lipid solvent for the extraction of most lipid material with the exception of a few complex lipid species(Schmid, 1973). In natural systems such as the North Saskatchewan river long chain fatty acids will be present as salts since the pH in the river is usually around 8.0. There will, at the same time, be hydrophobic associations of the long chain fatty acids

with the cell walls of procaryctes and the cell membranes of eucaryotes. To determine if any long chain fatty acids were associated with cells a sample of the Edmonton sewage effluent was filtered through a 0.45u cellulose filter(Millipore Corp. Bedford, Mass. U.S.A.) to remove most procaryotic and eucaryotic cells. Chloroform-methanol lipid extracts were made from acidified(pH 1.0) filtered and non-filtered sewage effluent samples. Separatory funnel and a reflux extraction techniques were compared for lipid recovery. The separatory funnel partitioning was done using three 20 ml changes of solvent followed by the pooling of the lower chloroform layer. The pooled chloroform layers were dried under a stream of nitrogen. The reflux technique was carried out in a 500 ml round bottom flask connected to a water jacketed condenser. To observe lipid recovery, the filtered and unfiltered samples were introduced into 2 separate flasks along with 4 times the volume of solvent and boiling chips. The samples to be refluxed were heated for 3 hours at 61°C followed by partitioning in a separatory funnel. The water layer was washed with 3 changes of the solvent and the lower layers were pooled and dried as previously described. All samples were assayed in duplicate for lipid material using a dichromate assay procedure.

II. Dichromate assay procedure for lipid material.

The method of Saito and Sato(1966) for the analysis of lipid material was used with a slight modification. That is, samples were concentrated by flash evaporator due to their low lipid concentration before solvent extraction.

The nitrogen dried extracts obtained from the extraction procedures were heated at 85°C for 5 minutes in the presence of a dichromate reagent (potassium dichromate 1 g, water 10 ml and concentrated sulphuric acid 190 ml). The solution was cooled to room temperature in a water bath and the absorbance at 670 nm was compared to that obtained with a standard of octadecanoic acid. A linear relationship between lipid content and the absorbance at 670 nm was found with concentrations up to 500 ug of lipid per assay tube(Saito and Sato, 1966).

III. Optimization of the reflux extraction method.

The relationship of reflux time to the maximum lipid release was determined. Aliquots of the refluxed sample were taken and assayed by the dichromate method. A 4 hour reflux was determined as being optimal and subsequently used throughout the river study.

IV. Bacterial metabolism of ^{14}C labelled long chain fatty acids.

FAU isolates were transferred from the maintenance cultures onto TYA agar incubated at 25°C. This was followed by transfer of freshly grown FAU isolates to TYB and incubation for 48 hours on a rotatory shaker(eccentricity 1 inch) at 300 rpm at 25°C. The absorbance of the culture determined at 600 nm using a 1 cm light path was found to be 1 unit. This was equivalent to about 10^9 P. aeruginosa cells per ml. Fifty ml of TYB plus 150 mg of ^{14}C carboxyl labelled sodium octadecanoate(specific activity 7.33×10^2 dpm/mg) were placed in a culture vessel. This was inoculated with 1 ml of the FAU cell suspension(from the TYB) and aerated with filter sterilized air for 72 hours. The vessel's effluent gases were passed through a gas washing bottle(Fisher scientific) containing saturated barium hydroxide. The carbon dioxide trapped as barium carbonate was released into phenethylamine as described previously. The resulting solution was counted in a liquid scintillation instrument(Isocap 300, Nuclear Chicago).

V. Ammonia and organic nitrogen analysis.

A standard macro-Kjeldahl procedure(American Public Health Association, 1971) was used to determine both

ammonia and organic nitrogen in all river and sewage effluent samples. The pH was adjusted to 7.4 with phoshate buffer in 300 ml river water samples or in 50 ml sewage effluent samples. The ammonia was distilled and trapped in a boric acid solution. To determine the organic nitrogen the samples were digested with sulphuric acid and potassium sulphate until all organic nitrogen was digested. The pH was raised and the ammonia distilled and trapped in boric acid. Residual boric acid was determined by titration with standard sulphuric acid.

VI. Carbon analysis.

The inorganic and total carbon content of all water samples were determined by the City of Edmonton Sewage Treatment Plant laboratory. The method used involved heating one sample at 150°C and measuring the amount of carbon dioxide produced. A second sample was heated to 950°C and the carbon dioxide measured. The amounts of carbon dioxide produced from the first sample was an estimate of the inorganic carbon and the second sample, the total carbon (Emery et al., 1971; Maier and Connell, 1974).

VII. Long chain fatty acid analysis.

The chloroform layer from the reflux extraction procedures was evaporated to dryness under a stream of nitrogen gas. The residue, dissolved in a small amount of fresh chloroform, was transferred to a 15 ml round bottom flask and then dried under nitrogen. A transesterification procedure was employed to produce the methyl esters of the long chain fatty acids. The reaction mixture consisted of the dried extract in the round bottom flask to which was added 2 ml of methanol(Fisher Scientific Co. certified ACS grade), 2 drops of concentrated sulphuric acid and a boiling stone. The flask and contents were heated to 55°C and gently refluxed for 2 hours using an air condenser. To remove the methanol and the esterified fatty acids after the reaction was complete, the flask was washed twice with water and once with n-hexane(Fisher Scientific Co. certified ACS grade). Any residual sulphuric acid present in the n-hexane layer was neutralized by washing with one 3 ml volume of 0.2M potassium bicarbonate and by two 5 ml water washes. All aqueous layers were discarded. The resulting n-hexane layer containing the methyl esters was dried by the addition of anhydrous sodium sulphate and the solvent evaporated under a stream of nitrogen(Given et al., 1973).

Prior to Gas Liquid Chromatographic(GLC) analysis,

the dried methyl ester extract was taken up in 100 ul of methylene chloride(Fisher Scientific Co. certified ACS grade). After the methyl ester extract was dissolved, 5 ul were injected into the GLC for analysis. Standards were run to determine retention times of the methyl esters. The standard used had an approximate concentration of 1 ug per 5 ul for each ester of the following mixture:

Methyl decanoate
Methyl dodecanoate
Methyl tetradecanoate
Methyl hexadecanoate
Methyl hexadecenoate
Methyl octadecanoate
Methyl octadecenoate
Methyl octadecadienoate
Methyl octadecatrienoate

For quantitative data the 2 following standards were used which were of analytical quality:

Methyl tetradecanoate at 1.25ug/5ul.
Methyl heptadecanoate at 10.00ug/5ul.

All fatty acid methyl esters were obtained from Applied Science Laboratories Inc. PO Box 440 State College, Penna., 16801, USA. A linear relationship as described by Ettre and Kabot(1963) existed between the relative peak areas and the concentrations of the latter 2 methyl esters(Methyl tetradecanoate and Methyl heptadecanoate)

on separate analysis.

The methyl esters were separated using a glass column containing a phase of 15% ethylene glycol succinate on a support of Chromosorb P, AW 100/120 mesh (Chromatographic Specialties, Brockville, Ontario, Canada). Initially, the column oven temperature was isothermal at 150°C for 8 minutes post injection. Temperature programming was started to increase at a rate of 32°C per minute until a final temperature of 190°C was reached. The 190°C temperature was maintained until the final methyl ester was eluted. The temperature of both the injector block and the hydrogen flame (FID) detector was 250°C. Carrier gas (nitrogen) flow rate was held at 60 ml/minute, hydrogen flow rate was at 39 ml/minute and air flow rate at 240 ml/minute. All gases were were dried before use. The column had a length of 6 feet and an internal diameter of 4mm. Hewlett-Packard (Hewlett-Packard, San Diego, California 92127, U.S.A.) instruments were used. The Gas Liquid Chromatograph model 5700A was coupled to a model 3370B integrator and a model 17503A single channel recorder.

VIII. Flow data and sewage dilution rate.

Discharge data for the North Saskatchewan river were obtained from the Water Survey of Canada (1973 and 1974) and data for the Edmonton domestic sewage

treatment plant from the City of Edmonton. The data obtained gave discharge rates in cubic feet per second and in million gallons per day for the river and the sewage treatment plant, respectively.

In order to observe any dilution effect the instantaneous dilution rate of the sewage effluent was calculated by the following equations:

Sewage dilution rate=SD/(RD x 0.539136)
where SD is sewage discharge in 10^6 gal/day and RD is the river discharge in cu ft/sec(CFS). The constant is derived from the transposition of CFS to 10^6 gal/day by the following equation.

CFSx6.24x60x60x24=gallonsx10^6/day.
where the second term is the conversion of cubic feet to gallons, the third term and fourth term converts seconds to hours and the fifth term converts hours to days.

The dilution rate is the instantaneous dilution rate based on the figures given which are average readings for the day in question and not necessarily the dilution rate at any particular time during the day of sampling.

D. STATISTICAL ANALYSIS OF DATA.

All sites were sampled in triplicate and each sample plated for bacterial ccunts in quintuplicate. The quintuplicate plate count data was subjected to the Nalimov test(Kaiser, 1971) which objectively removes data that is considered 'runaway' or not clustered at p=0.05. In sc doing, the mean becomes more clustered and has less variance due to the determinate error. This procedure then gives 3 means of the sample site bacterial ccunts. To obtain the overall mean bacterial count for a sample site the 3 means are tested by analysis of variance. The standard deviation is determined frcm the analysis of variance and indicates the randomness of the bacterial numbers found at the sample site.

All measured parameters were then interccmpared using the simple correlation coefficient(Sokal and Rohlf, 1969) tc determine the degree of relatedness between these parameters(correlation). The correlation coefficient(r) is determined in this procedure. If the r term is squared the coefficient of determination is thus derived. In effect this latter r squared term indicates that proportion of the total variability which was due to scme linear relationship existing between the 2 parameters in question. The remaining variability was

due to some unexplained factor(s) which is(are) usually called 'experimental error'.

RESULTS AND DISCUSSION

A. MICROBIOLOGICAL.

1. Utilization of long chain fatty acids by lipase producing P. aeruginosa ATCC 9027.

Losses of lipid material from TYB medium containing octadecanoate inoculated with P. aeruginosa ATCC 9027 are shown in Table 2. There was an absolute loss of 180 mg of lipid material over a 5 day period. Background TYB medium lipid levels were subtracted from the lipid levels of the octadecanoate containing TYB. Recovery of added lipid material was a problem in that only 90% was recovered from the zero time sample. That is 250 mg/l were added and only 225 mg/l were recovered. These results were based on an average of duplicate assays. The data indicates that the disappearence of lipid material started within the first day. Evidence is given in Table 3 to show that P. aeruginosa ATCC 9027 does take up and metabolize the long chain fatty acids. The presence of ^{14}C labelled fatty acids in TYB result in the production of ^{14}C labelled carbon dioxide when inoculated with the microorganism. During the 5 day incubation period 50.7% of the ^{14}C label was recovered as ^{14}C labelled carbon dioxide. Therefore the lipase producing P. aeruginosa ATCC 9027 can hydrolyze and utilize long chain fatty acids. This microorganism was

TABLE 2

EVIDENCE INDICATING THAT LIPASE PRODUCING PSEUDOMONAS AERUGINOSA ATCC 9027 CAN METABOLIZE LONG CHAIN FATTY ACIDS

Treatment	Incubation Days 0	1	2	3	4	5
	Lipid Content of Medium mg/l					
Inoculated - Octadecanoate - 250 mg/l	225	175	ND	130	ND	45
Inoculated - No fatty acid additions	0	0	ND	0	ND	0

Incubation conditions were 25°C on a rotatory shaker at 300 rpm
ND = Not determined

TABLE 3

EVIDENCE INDICATING THAT LIPASE PRODUCING PSEUDOMONAS AERUGINOSA ATCC 9027 CAN METABOLIZE ^{14}C LABELLED LONG CHAIN FATTY ACIDS

	Total $^{14}CO_2$ Production from Uniformly ^{14}C Labelled Hexadecanoate and Octadecanoate DPM					
Inoculated	10	606	ND	806	ND	1112
Uninoculated	13	15	ND	18	ND	ND

Incubation conditions were 25°C on a rotatory shaker at 300 rpm

Specific activity for the fatty acids were 12.5 DPM/mg. The fatty acid concentration was 0.3% w/v.

Total culture volume = 1 litre

Label recovery = 50.7%

ND = Not determined

therefore used as a positive control species.

II. Development of a method for direct enumeration of long chain fatty acid utilizing bacteria.

Many attempts using rhodamine B:uranyl acetate complex dissolved in various organic solvents failed to detect differences in long chain fatty acid concentration within solid medium after the reagent was sprayed on the agar surface. The rhodamine B:uranyl acetate reaction was tested using only a long chain fatty acid solution giving similar results to those described for the spectrophotometric method as outlined by Mackenzie et al. (1967).

A second method tried was a modification of that of Sulya and Smith (1960) involving the use of an acidic solution of protoporphyrin IX. This reagent forms a UV light detectable complex with long chain fatty acids. However, no differential reaction was obtained when TYA agar containing sodium ocatadecanoate inoculated with the test micrcorganism was sprayed with the reagent. Agar appears to be an excellent quenching agent since the reaction produced with fatty acids when spotted onto chromatography paper was similar to that reported by Sulya and Smith (1960).

It was observed that a TYA agar plate containing

1.0% sodium octadecanoate and inoculated with P. aeruginosa ATCC 9027 when dried, left a clear area directly under the colony. Similar results could be obtained in 10-14 days incubation at 25 degrees with thin agar plate. Typical results using this method are shown in Plate 5. The top photograph shows the intact growing colonies on TYA agar plus 1.0% sodium octadecanoate before their removal with the buffered salts solution containing 1% Triton X-100. The lower photograph shows the appearence of the medium after the colonies have been removed.

Thus long chain fatty acid utilizing bacteria(FAU) can be detected and counted by growth on thin TYA agar plates containing 1.0% sodium octadecanoate. The effect of the long chain fatty acid concentration in the solid medium on the sensitivity of the assay was tested. Data from a sample of Vinca Bridge river water(Table 4) shows a comparison of the numbers of FAU growing and the number clearing the TYA+FA agar compared with the TYA total count control. It was observed that the sodium octadecanoate flocculated when at a concentration of less than 0.3% such that its concentration varied throughout the medium. At concentrations greater than 0.3% the agar appeared homogeneous. The uniform distribution of the long chain fatty acid throughout the plate was due in part to the 80°C pouring temperature.

PLATE 5

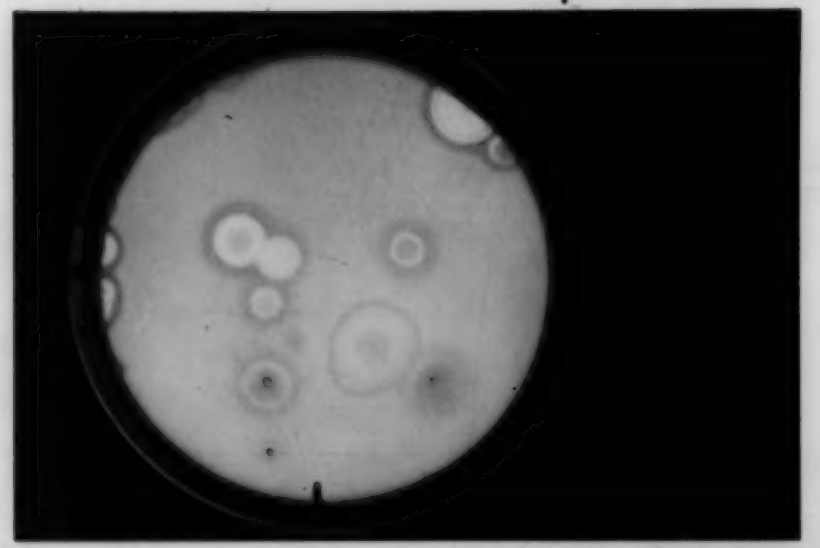
PHOTOGRAPHS SHOWING THE EFFECT OF FAU

ON TYA AGAR CONTAINING 0.3% SODIUM OCTADECANOATE

AT pH 8.0

UPPER - GROWING COLONIES

LOWER - EFFECT OF FAU ON THE TYA+FA AGAR



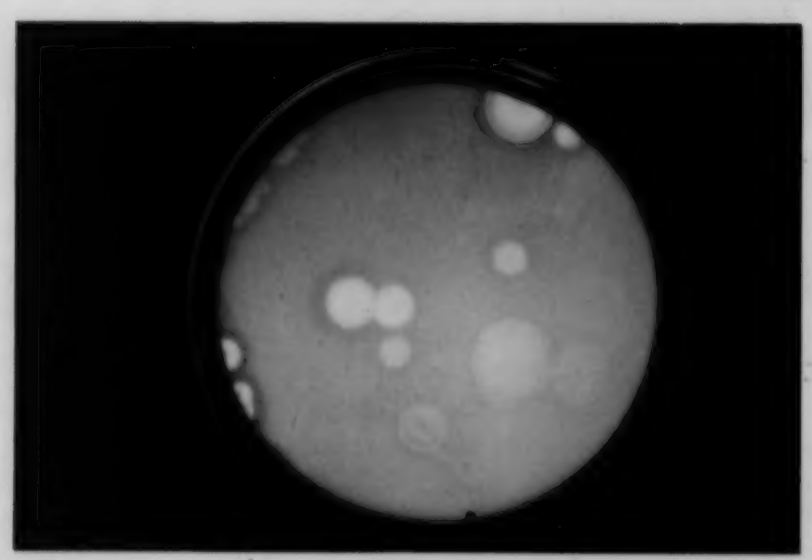


TABLE 4

EFFECT OF THE LONG CHAIN FATTY ACID CONCENTRATION IN SOLID MEDIUM ON THE NUMBERS OF FATTY ACID UTILIZING BACTERIA

% Sodium octadecanoate w/v	Colony Status	Number of Colonies per plate**	% of Total Count*
0.01	Growing	43.8	67.1
	Clearing	cannot detect clearing	
0.1	Growing	29.8	45.6
	Clearing	cannot detect clearing	
0.3	Growing	25.6	39.3
	Clearing	20.8	31.9
0.5	Growing	23.0	35.3
	Clearing	18.6	28.5
0.8	Growing	20.6	31.6
	Clearing	17.0	26.1
1.0	Growing	20.4	31.3
	Clearing	17.3	26.5

*The total FAU count of the sample expressed as a percentage of the total bacterial count of that sample which was 65.2 colonies

**The total FAU count

The data in Table 4 indicates that as the sodium octadecanoate concentration increases both the numbers growing on and the numbers clearing TYA+FA decrease at a linear but differing rate. That is to say, as the long chain fatty acid concentration increases the number of bacteria clearing the medium and the number of bacteria growing on the medium tend toward the same number. Therefore 0.3% sodium octadecanoate appears to be the optimal long chain fatty acid concentration for sensitivity.

Although there is a slight difference in the numbers clearing and growing on this medium it was subsequently found that the difference was not statistically significant. Table 5 contains data showing that at a p=0.05 there is overlap of the standard deviation of the mean of the clearing count and the mean of the growing count.

It appears from the data presented in this section that a TYA medium plus 0.3% sodium octadecanoate at pH 8.0-8.3 is the optimum medium for the direct enumeration of long chain fatty acid utilizers. In addition there is no significant difference between the number of colonies clearing the medium and the number that are growing on the medium since there is overlap of the standard deviation. Therefore in future studies the colonies growing were taken as equivalent to the FAU

TABLE 5

COMPARISON OF COUNTS OF LONG CHAIN FATTY ACID UTILIZING BACTERIA

Sample Date	Sample Site	Bacterial Count ±SD* / ml Growing	Clearing
7/11/73	156 Street	$(4.9 \pm 0.4) \times 10^1$	$(4.2 \pm 0.8) \times 10^1$
7/11/73	Sewage Effluent	$(1.6 \pm 0.7) \times 10^4$	$(1.5 \pm 0.7) \times 10^4$
7/11/73	Vinca Bridge	$(7.8 \pm 1.6) \times 10^1$	$(7.8 \pm 2.3) \times 10^1$

*S.D. = Standard Deviation

count. A similar conclusion cannot be drawn for the sodium octadecanoate concentration of less than 0.3% since clearing cannot be detected at these low long chain fatty acid concentrations. Therefore this technique will not differentiate the long chain fatty acid tolerant bacteria from the FAU.

III. Surface water bacterial counts throughout one sample year at 156 St., Edmonton domestic sewage effluent and Vinca bridge sample sites.

The total bacterial count at 156 St. varies as expected during the sample year in that the winter months show a decreasing count which drops to a level of $1.4-3.0 \times 10^3$ microorganisms per ml of surface water. Although the latter counts are for water under total ice cover conditions little difference is seen between them and those obtained on 6/9/73 (Tables 6 and 7). During breakup around 22/4/74 the total count initially increases about 200-fold and reaches a 400-fold value on 29/4/74. Of further interest in this respect is the fact that on 22/4/74 the river discharge was greater than 8.2×10^{10} litres per day whereas on 29/4/74 it was down to a discharge of slightly greater than 5.7×10^{10} litres per day (Table 31). This may indicate a possible growth of the population present during this period since the microbiological load is twice as great as that

TABLE 6

BACTERIAL COUNTS OF NORTH SASKATCHEWAN RIVER WATER AT 156 STREET THROUGHOUT THE SAMPLE YEAR

Sample Date	Bacterial Count ± 1SD* per ml		
	Total Count	Pigmented Count	FAU** Count
6/9/73	$2.3 \pm 0.3 \times 10^3$	$1.2 \pm 0.1 \times 10^3$	$2.5 \pm 0.2 \times 10^1$
7/11/73	$3.0 \pm 0.2 \times 10^3$	$1.2 \pm 0.1 \times 10^3$	$4.2 \pm 0.8 \times 10^1$
24/1/74	$1.4 \pm 0.1 \times 10^3$	$5.1 \pm 0.3 \times 10^2$	$4.5 \pm 0.2 \times 10^1$
2/4/74	$2.7 \pm 0.9 \times 10^3$	$1.1 \pm 0.5 \times 10^3$	$3.0 \pm 0.3 \times 10^1$
22/4/74	$4.1 \pm 0.2 \times 10^5$	$1.7 \pm 0.3 \times 10^5$	$1.1 \pm 0.1 \times 10^4$
29/4/74	$9.3 \pm 0.2 \times 10^4$	$3.9 \pm 0.9 \times 10^4$	$1.4 \pm 0.2 \times 10^3$
21/5/74	$1.1 \pm 0.1 \times 10^4$	$4.6 \pm 0.4 \times 10^3$	$2.0 \pm 0.2 \times 10^2$
15/7/74	$4.9 \pm 0.2 \times 10^4$	$2.0 \pm 0.2 \times 10^4$	$1.0 \pm 0.2 \times 10^3$

*SD = Standard Deviation

**FAU = Long Chain Fatty Acid Utilizer

of the latter date and the discharge is about one half. By the time mid-summer is reached the total counts are back to a level that had been previously found at 156 St. and Vinca bridge sample sites(Coleman et al., 1974).

In naturally occurring water, pigmented bacteria constitute a large fraction of the total bacteria(Jones, 1970;Coleman et al., 1974). The pigmented bacterial count represents a relatively constant fraction of the total count at 156 St.(Table 6). In general the FAU count is about 1-5% of the total bacterial population at this site. These results compare favourably with those results found previously for water samples taken during the winter(Coleman et al., 1974).

The sewage effluent counts(Table 7) show little variation in the numbers of bateria present. The total count is relatively constant, showing a level of around $1x10^6$ per ml, with the exception of the sample taken on 24/1/74 where the count was greater than $1x10^7$ per ml. This situation occurred during a pulse loading problem where the attached growth present at the aeration step sloughed off and this resulted in the high bacterial counts in the sewage effluent. High counts persisted for about 2 weeks until the system equilibrated and could subsequently handle the new input. The pulse loading occurred as a result of the wastes from packing

houses being re-routed to the domestic sewage treatment plant(Table 7). The observed constant numbers of bacteria present in the sewage effluent are probably dependent to a large extent upon the more or less constant temperature and nutrient concentration of the system. The maximum temperature variation was 4°C over the year(Table 31). Pigmented bacteria constitute a lesser fraction(30%) of the total count in the sewage effluent as compared to the river water at 156 St. sample site. The FAU bacterial numbers present in the sewage effluent are always 2% of the total count. This may suggest that 2% of the total bacterial cells present have a secondary ability to utilize long chain fatty acids.

There appears to be approximately a 10 fold increase in the total count in the Vinca bridge samples as compared to the 156 St. samples throughout the sample year. This increase in bacterial numbers is possibly due to the effect of the sewage effluent adding fresh nutrients and promoting growth of bacteria in the river. There is one obvious exception which occurred at breakup(22/4/74) when all sites sampled show the same magnitude of counts. The level at 156 St. is slightly higher that that found at Vinca bridge(Tables 6,7 and 8). This higher count is probably due to a decrease in velocity of the river water at the latter site with

TABLE 7

BACTERIAL COUNTS OF EDMONTON DOMESTIC SEWAGE EFFLUENT THROUGHOUT THE SAMPLE YEAR

Sample Date	Bacterial Count±1SD* per ml.		
	Total Count	Pigmented Count	FAU** Count
6/9/73	$1.7\pm0.2\times10^{6}$	$1.0\pm0.1\times10^{6}$	$1.2\pm0.8\times10^{5}$
7/11/73	$9.5\pm1.7\times10^{5}$	$2.1\pm0.4\times10^{5}$	$1.5\pm0.7\times10^{4}$
24/1/74	$1.1\pm0.4\times10^{7}$	$2.8\pm0.7\times10^{6}$	$1.2\pm0.4\times10^{5}$
2/4/74	$4.1\pm0.4\times10^{5}$	$8.3\pm2.5\times10^{4}$	$6.6\pm3.0\times10^{3}$
22/4/74	$6.8\pm0.5\times10^{5}$	$2.3\pm0.4\times10^{5}$	$1.5\pm0.2\times10^{4}$
29/4/74	$8.2\pm0.8\times10^{5}$	$2.8\pm0.2\times10^{5}$	$1.7\pm0.2\times10^{4}$
21/5/74	$2.3\pm0.2\times10^{6}$	$5.6\pm1.8\times10^{5}$	$4.4\pm0.5\times10^{5}$
15/7/74	$5.4\pm0.5\times10^{5}$	$1.9\pm0.4\times10^{5}$	$1.6\pm0.01\times10^{4}$

*SD = Standard Deviation

**FAU = Long Chain Fatty Acid Utilizer

TABLE 8

BACTERIAL COUNTS OF NORTH SASKATCHEWAN RIVER WATER AT VINCA BRIDGE THROUGHOUT THE SAMPLE YEAR

Sample Date	Bacterial Count ± SD* per ml		
	Total Count	Pigmented count	F.A.U.** Count
6/9/73	$9.2 \pm 0.7 \times 10^4$	$7.6 \pm 0.5 \times 10^3$	$1.7 \pm 0.5 \times 10^3$
15/11/73	$3.7 \pm 1.1 \times 10^4$	$7.4 \pm 0.6 \times 10^3$	$7.8 \pm 1.6 \times 10^1$
27/1/74	$2.2 \pm 0.3 \times 10^4$	$4.5 \pm 0.3 \times 10^3$	$2.9 \pm 0.3 \times 10^2$
2/4/74	$4.2 \pm 0.3 \times 10^4$	$1.1 \pm 0.1 \times 10^4$	$3.4 \pm 0.2 \times 10^2$
22/4/74	$3.7 \pm 0.1 \times 10^5$	$1.2 \pm 0.2 \times 10^5$	$1.3 \pm 0.2 \times 10^4$
29/4/74	$2.9 \pm 0.3 \times 10^5$	$1.5 \pm 0.4 \times 10^5$	$2.3 \pm 0.3 \times 10^3$
21/5/74	$6.3 \pm 0.2 \times 10^4$	$1.8 \pm 0.1 \times 10^4$	$7.5 \pm 0.2 \times 10^2$
15.7.74	$8.5 \pm 0.7 \times 10^4$	$3.5 \pm 0.1 \times 10^4$	$2.4 \pm 0.1 \times 10^3$

*SD = Standard Deviation

**FAU = Long Chain Fatty Acid Utilizer

subsequent sedimentation of the bacterial cells along with the particulate material. At Vinca bridge the river channel is wider than at 156 St. and thus the velocity of flow lower. These increases in total count are expected in the river because of the runoff water adding nutrients and cells. The slightly lower increase in the total count observed in the sewage effluent is possibly a result of high hydraulic loading due to thawing conditions resulting in a decreased sewage detention time. The similarity of total bacterial counts at the 3 sample sites(22/4/74) may reflect the average total bacterial count of runoff water. This situation is short lived since the river rapidly returns to the previously observed differences in bacterial counts between upstream and downstream Edmonton.

IV. The production of ^{14}C labelled carbon dioxide from ^{14}C labelled octadecanoate by FAU.

Generally, all of the FAU isolates tested could produce in excess of 2500 DPM of ^{14}C labelled carbon dioxide from ^{14}C labelled sodium octadecanoate. The method used yielded qualitative results since the inoculation was not standardized with regard to cell numbers. The results in Table 9 indicate that isolated FAU's can utilize octadecanoate when compared to an uninoculated control. There is only an inference here

TABLE 9

PRODUCTION OF ^{14}C LABELLED CARBON DIOXIDE FROM ^{14}C LABELLED OCTADECANOATE* BY SOME SELECTED F.A.U. ISOLATES

Isolate Number	Sample Site	Genus or Family	^{14}Carbon Dioxide Production DPM/3 Days
19	156 St.	Pseudomonas	5198
23	Sewage Effluent	Enterobacteriaceae	7558
27	Sewage Effluent	Enterobacteriaceae	6235
28	Sewage Effluent	Enterobacteriaceae	2640
34	Sewage Effluent	Aeromonas	5043
62	Sewage Effluent	Aeromonas	18408
88	Vinca Bridge	Aeromonas	17949
98	Vinca Bridge	Alcaligenes	8966
102	Sewage Effluent	Alcaligenes	6014
172	Sewage Effluent	Enterobacteriaceae	13959
177	Sewage Effluent	Alcaligenes	15997
181	Sewage Effluent	Enterobacteriaceae	14188
183	Sewage Effluent	Pseudomonas	34290
186	Sewage Effluent	Pseudomonas	38708
187	Sewage Effluent	Pseudomonas	5235
192	Vinca Bridge	Pseudomonas	4690
197	Vinca Bridge	Enterobacteriaceae	4590
234	Vinca Bridge	Pseudomonas	10997
242	Vinca Bridge	Pseudomonas	8395
Uninoculated Control			19

*Specific activity = 733 dpm/mg. (1.1×10^5 dpm added and approximately 10% of the label recovered as ^{14}C labelled carbon dioxide)

that all the FAU's isolated during the entire study can utilize octadecanoate since only 5.1% of the total were tested. However this list of genera is a representative spectrum of those isolated.

V. Classification of FAU bacterial isolates from the 156 St., sewage effluent and Vinca bridge sample sites.

According to the 8th edition of Bergey's Manual of Determinative Bacteriology the genus Achromobacter does not exist. The members of this genus have been primarily distributed amongst the genera Acinetobacter and Alcaligenes. The tests used would not separate members of the genus Acinetobacter from the genus Xanthomonas since they both may be oxidase negative, non-pigmented and oxidatively metabolize glucose. This was considered and any microbial isolate with the above characteristics were placed in the genus Acinetobacter since Xanthomonas sp. are usually considered to be plant pathogens.

Table 10 contains the taxonomic data of the long chain fatty acid utilizing isolates from the 156 St. sample site throughout the sample year. The genus Pseudomonas predominates with the exception of the sample taken on 15/7/74. In this sample there are greater numbers of both the family Enterobacteriaceae and the genus Aeromonas. A few isolates of the genus

TABLE 10

CHARACTERIZATION OF LONG CHAIN FATTY ACID UTILIZING ISOLATES FROM THE 156 ST. SAMPLE SITE OVER THE SAMPLE YEAR

Sample Date	Family or Genera as a Percentage of the Total F.A.U.*						
	Pseudomonas	Enterobacteriaceae	Aeromonas	Acinetobacter	Alcaligenes	Micrococcus	Unclassified
6/9/73	53.9	16.6	22.1	5.4	2.7	-	-
14/11/73	63.2	5.3	26.3	35.3	-	-	-
24/1/74	42.8	28.6	28.6	-	-	-	-
2/4/74	76.2	-	14.2	-	4.8	-	4.8
22/4/74	86.7	-	-	-	13.3	-	-
29/4/74	78.6	-	14.3	7.1	-	-	-
21/5/74	91.7	-	8.3	-	-	-	-
15/7/74	25.0	37.5	-	-	-	-	-

F.A.U. = Long Chain Fatty Acid Utilizer

Acinetobacter were present in samples taken on 9/9/73, 11/11/73 and 29/4/74.

The sewage effluent data shows few, if any trends(Table 11). The genus Pseudomonas appears to dominate during high sewage treatment plant flow(Table 31) which occurs in early April. Generally the family Enterobacteriaceae predominates along with the genus Aeromonas. Both groups tend to be diluted almost to extinction or disappear during high hydraulic load on the sewage treament plant occurring in spring thaw conditions(April). Enterobacteriaceae sp. and Aeromonas sp. are facultatively anaerobic and they can survive the treatment plant's anaerobic phase whereas Pseudomonas sp. would be tend to be eliminated through competition. Under conditions of high flow rate however the retention time would be reduced in the anaerobic portion of treatment thus allowing Pseudomonas sp. to survive. Alcaligenes sp. are also present at low levels throughout the sample year with some exceptions. They were non-detectable in samples taken on 11/11/74 and 15/7/74 although representing a major fraction on 21/5/74. It would appear that this genus is less important than that of Aeromonas sp. or the family Enterobacteriaceae in the sewage system as FAU. The genus Acinetobacter sp. also appears to be a minor component of the FAU in the sewage effluent as they are

TABLE 11

CHARACTERIZATION OF LONG CHAIN FATTY ACID UTILIZATION ISOLATES FROM THE SEWAGE EFFLUENT SAMPLE SITE OVER THE SAMPLE YEAR

Sample Date	Family or Genera as a Percentage of the Total F.A.U.*						
	Pseudomonas	Enterobacteriaceae	Aeromonas	Acinetobacter	Alcaligenes	Micrococcus	Unclassified
6/9/73	23.9	46.1	29.5	8.1	9.1	-	-
14/11/73	-	64.3	32.1	-	-	3.6	-
24/1/74	9.1	45.5	27.2	-	18.2	-	-
2/4/74	36.3	35.5	15.6	8.0	4.6	-	-
22/4/74	52.9	29.4	-	5.9	11.8	-	-
29/4/74	8.4	33.3	33.3	-	25.0	-	-
21/5/74	6.3	37.5	25.0	-	31.2	-	-
15/7/74	12.5	50.0	37.5	-	-	-	-

*FAU = Long Chain Fatty Acid Utilizer

never present at concentrations greater than 8.1%(9/9/73).

All microbial genera found at the Vinca bridge sample site suggest that the water at this site is primarily a mixture of the sewage effluent and river water from the 156 St. site(Table 12). Generally the content of the genus Pseudomonas is fractionally less than that found in either the sewage effluent or 156 St. sample. The difference between Vinca bridge and 156 St. samples is much less than that between the sewage effluent and Vinca bridge. There is little change in the fractional distribution of the FAU population as the river passes through Edmonton although the absolute numbers do increase. The family Enterobacteriaceae are either absent at Vinca bridge or are reduced in fraction as compared to the sewage effluent, indicating either dilution or killing after discharge of the sewage effluent to the river. The genus Aeromonas on the other hand shows little fractional change when comparing the sewage effluent with Vinca bridge or 156 St., possibly members of this genus are adaptive in the 3 differing sample sites studied. Other lesser represented groups show considerable variation indicating that they are possibly less important as FAU.

There is a sewage effluent dilution factor to be considered where the sewage effluent enters the North

TABLE 12

CHARACTERIZATION OF LONG CHAIN FATTY ACID UTILIZING ISOLATES FROM THE VINCA BRIDGE SAMPLE SITE OVER THE SAMPLE YEAR

Sample Date	Family or Genera as a Percentage of the Total F.A.U.*						
	Pseudomonas	Enterobacteriaceae	Aeromonas	Acinetobacter	Alcaligenes	Micrococcus	Unclassified
6/9/73	44.7	25.6	25.3	11.9	7.0	-	-
14/11/73	-	53.8	23.1	7.7	15.4	-	-
24/1/73	30.8	30.8	30.8	-	7.6	-	-
2/4/74	68.2	13.6	-	13.6	4.6	-	-
22/4/74	82.3	11.8	-	5.9	-	-	-
29/4/74	45.4	-	27.3	13.6	13.7	-	-
21/5/74	27.3	18.2	36.3	9.1	9.1	-	-
15.7/74	35.0	5.3	30.0	5.0	25.0	-	-

*FAU = Long Chain Fatty Acid Utilizer

Saskatchewan river. A calculation of the dilution effect on the numbers of Enterobacteriaceae at Vinca bridge indicates that the numbers have only been decreased by the dilution factor. There is no growth of this microbial group during the spring and summer months, however during late fall and the winter months a slight but not significant decrease in numbers occurs. This agrees with previous observations(Coleman et al., 1974) that the E. coli count peaked in the river directly after Edmonton but was followed by fairly rapid decline in numbers further downstream.

VI. Classification of the long chain fatty acid non-utilizing bacterial isolates from the 156 St., sewage effluent and Vinca bridge sample sites.

The first 3 sample dates are not entered in Tables 13, 14 and 15 since at that time the classification of the long chain fatty acid non-utilizing microorganisms was not carried out. On later samples all such isolates were placed in stock culture for classification. The genus Flavobacterium is by far the most prominant member of this group. Since special medium was not used to check for gliding motility there is a possibility of confusing the genus Cytophaga with the genus Flavobacterium. Although determination of flagella arrangement would have aided in their separation it was

TABLE 13

CHARACTERIZATION OF LONG CHAIN FATTY ACID NON UTILIZING BACTERIAL ISOLATES FROM THE 156 ST. SAMPLE SITE

Sample Date	Genera as a Percentage of Total F.A.N.U.*						
	Flavo-bacterium	Acineto-bacter	Alcaligenes	Aeromonas	Micrococcus	Pseudomonas	Others or Unknown
2/4/74	30.0	35.0	20.0	5.0	10.0	-	-
22/4/74	60.0	20.0	5.0	-	-	5.0	5.0** 5.0***
29/4/74	100.0	-	-	-	-	-	-
21/5/74	60.0	13.3	-	-	-	13.3	13.3***
15/7/74	38.4	-	23.1	7.7	-	23.1	7.7***

* = Long Chain Fatty Acid Utilizer

** = Arthrobacter sp.

*** = Cytophaga sp.

TABLE 14

CHARACTERIZATION OF LONG CHAIN FATTY ACID NON UTILIZING BACTERIAL ISOLATES FROM THE SEWAGE EFFLUENT SAMPLE SITE

Sample Date	Genera as a Percentage of Total F.A.N.U.*						
	Flavo-bacterium	Acinete-bacter	Alcaligenes	Aeromonas	Micrococcus	Pseudomonas	Others or Unknown
2/4/74	20.0	20.0	-	-	60.0	-	-
22/4/74	30.0	30.0	-	-	-	25.0	5.0,5.0*** 5.0**
29/4/74	44.4	7.4	37.0	7.5	-	-	3.7**
21/5/74	58.3	8.3	25.0	-	8.4	-	-
15/7/74	33.3	-	53.4	13.3	-	-	-

*F.A.N.U. = Long chain fatty acid non utilizer

**Mycobacterium

***Cytophaga

TABLE 15

CHARACTERIZATION OF LONG CHAIN FATTY ACID NON UTILIZING ISOLATES FROM THE VINCA BRIDGE SAMPLE SITE

Sample Date	Genera as a Percentage of the Total F.A.N.U.*						
	Flavo bacterium	Acinetobacter	Alcaligenes	Aeromonas	Micrococcus	Pseudomonas	Others or unknown
2/4/74	33.0	-	55.0	-	-	5.6	5.5
22/4/74	52.2	8.7	21.7	-	4.4	-	13.0**
29/4/74	53.9	15.4	21.9	4.4	-	4.4	-
21/5/74	55.6	22.2	22.2	-	-	-	-
15/7/74	66.7	-	11.1	11.1	-	11.1	-

* F.A.N.U. = Long chain fatty acid non utilizer

** Cytophaga

discontinued because of excessive numbers of isolates. However in obvious cases where cytophaga showed typical colonial formation due to gliding motility the isolate was then classified as a cytophaga.

The sample site at 156 St. (Table 13) generally shows at least an equal distribution of different genera of non-utilizers when compared to those found at Vinca bridge (Table 15). Generally, a greater fraction of pigmented types were present in river water as compared to those present in the sewage effluent (Table 14). The genus Alcaligenes again represents a noticeable but minor fraction of the population as it does in the case of the utilizers. An unexpected observation was the fact that the genera Aeromonas, Pseudomonas and Acinetobacter are also present as long chain fatty acid non-utilizers. This was unusual since the FAU include, as a major fraction, representatives of these 3 genera (Tables 10, 11 and 12). Micrococcus sp. are a greater proportion of the FANU as compared to those present in the FAU. One isolation of the genus Arthrobacter was made on 22/4/74. This identification was confirmed by following the life cycle. Other pigmented varieties could be of this genus but were not specifically tested.

VII. Comparison of bacterial counts in the sediment with those of the surface water at all river sampling sites.

Since sediment samples are only obtained with great difficulty during ice cover conditions sediment analyses were limited to the summer period. Data from the 156 St. and Vinca bridge sites suggest that the total count is a 1000-fold higher in the sediment as compared to the surface water when expressed on a volume basis. This is similar to the results found previously(Jannasch, 1956;Coleman et al., 1974). The source sample site however showed only a 50-fold increase in the sediment as compared to the surface water(Table 16). These results are similar to those found previously(Coleman et al., 1974). During sampling of the sediment at 156 St. it became obvious that there were 2 types of sediment. One consisting of small rocks, gravel, sand and silt(SED I) and another consisting mostly of coarse sand with some silt(SED II). The data in Table 16 clearly shows that there is almost a 10-fold difference between the counts in SED II and SED I. The larger rocks present in SED I were almost entirely covered in algal growth and other attached material.

Vinca bridge sediment is grossly comparable to the sediment of SED II of 156 St. The sediment at Vinca bridge contained a 1000-fold increase in bacterial

TABLE 16

SEDIMENT AND WATER BACTERIAL COUNTS FROM ALL RIVER SITES

Sample Date	Sample Location	Sample Type	Viable Count /ml ± 1 S.D. Total Count	Pigmented Count	FAU Count
3/6/74	156 St.	Water	$9.4\pm0.5\times10^{3}$	$3.6\pm0.06\times10^{3}$	$1.3\pm0.2\times10^{2}$
3/6/74	156 St.	Sed.* I	$4.6\pm0.1\times10^{5}$	$1.8\pm0.1\times10^{5}$	$6.0\pm0.4\times10^{3}$
3/6/74	156 St.	Sed.* II	$3.4\pm0.3\times10^{6}$	$1.3\pm0.1\times10^{6}$	$1.6\pm0.2\times10^{4}$
4/6/74	Vinca Bridge	Water	$7.5\pm0.3\times10^{4}$	$1.8\pm0.1\times10^{4}$	$1.2\pm0.2\times10^{3}$
4/6/74	Vinca Bridge	Sed.**	$5.2\pm0.1\times10^{7}$	$1.0\pm0.1\times10^{7}$	$4.3\pm1.0\times10^{5}$
25/6/74	Source	Water	$1.8\pm0.4\times10^{2}$	$5.7\pm0.1\times10^{1}$	$9.3\pm0.2\times10^{0}$
25/6/74	Source	Sed.***	$8.8\pm0.9\times10^{3}$	$3.1\pm0.5\times10^{3}$	$4.2\pm0.4\times10^{2}$

* Sed.I = Sediment consisting of small rocks, gravel and silt
Sed. II = Sediment consisting entirely of coarse sand and some silt

** Sed. = Sediment consisting of fine sand, silt and organic material

*** Sed. = Sediment consisting of large rocks, small rocks and glacial flour

SD = Standard Deviation

FAU = Long Chain Fatty Acid Utilizer

counts as compared to the surface water. This indicates either a larger surface area to volume ratio in this sediment or that the sediment could support a larger bacterial population as compared with the sediment of SED II at 156 St.

The sediment at the source had a coarse rock and glacial flour composition. Total count data from this location suggests that there is a 50-fold increase in bacterial numbers in the sediment as compared to the water. There is some similarity in the ratio of the sediment to water counts here with those found at the 156 St. sample site (SED I).

Although the PVC pipe used in sampling the sediment minimized the surface water contamination there may have been some seepage and some dilution and thus contamination of sediment bacterial species with water species. Contamination, however, was difficult to determine.

VIII. Classification of both the long chain fatty acid utilizers and the non-utilizers found in sediment and water at all river sites.

Pseudomonas sp. predominate in the sediment at the source of the North Saskatchewan river and comprise the entire population of FAU in the water at this site (Table

17). Other genera present in the sediment at this location are Acinetobacter and Alcaligenes comprising the remaining 10% and 20% of the total FAU population, respectively. Pseudomonas sp. may be selected for by the high aeration and low temperature that are characteristic of this section of the river.

A different pattern is found in the water at 156 St. where the genus Aeromonas predominates closely followed by Pseudomonas sp. There are low levels of members of the family Enterobacteriaceae and the genus Alcaligenes found here. The sediment at 156 St., on the other hand, generally contains higher numbers of the facultative anaerobes such as Aeromonas sp. and other Enterobacteriaceae. The black color of the sediment observed at the time of sampling is presumably due to the action of sulphate reducers. In general, the genus Acinetobacter are not found as long chain fatty acid utilizers in sediments except in the source sediment sample(Tables 7 and 9).

The water at Vinca bridge contains the same representation of genera that are found at 156 St. but with a slight increase in the numbers of Aeromonas sp. at the apparent expense of Pseudomonas sp. Data from the sediment on the other hand at this location presents a totally different picture in that the FAU present are entirely facultative anaerobes(40% Aeromonas sp. and

TABLE 17

CHARACTERIZATION OF LONG CHAIN FATTY UTILIZING BACTERIAL ISOLATES FROM SEDIMENT AND WATER SAMPLES

Sample Data	Sample Type and Location	Family or Genera as a Percentage of the total F.A.U.*						
		Pseudomonas	Enterobacteriaceae	Aeromonas	Acinetobacter	Alcaligenes	Micrococcus	Unknown
3/6/74	156 St. Water	33.4	11.1	44.4	-	11.1	-	-
3/6/74	156 St. Sed I **	25.0	25.0	50.0	-	-	-	-
3/6/74	156 St.*** Sed II	50.0	25.0	12.5	12.5	-	-	-
4/6/74	Vinca Br. Water	10.0	10.0	70.0	-	10.0	-	-
4/6/74	Vinca Br. Sed +	-		60.0	-	-	-	-
24/6/74	Source Water	100	-	-	-	-	-	-
24/6/74	Source Sed.++	70	-	-	10	20	-	-

* = Long chain fatty acid utilizer
** = Sediment I (See Table 16)
*** = Sediment II (See Table 16)
\+ = Sediment - course sand, fine sand, silt, organic material, sulphides
++ = Large rocks, glacial flour

60% Enterobacteriaceae). This possibly reflects the effects of the Edmonton sewage treatment plant effluent upon the river bacterial population.

As found in water at other sampling times, the genus Flavobacterium predominate at the pre-Edmonton sample sites in both the sediment and the water. They represent the majority of the long chain fatty acid non-utilizers (Table 18). Alcaligenes sp. and Acinetobacter sp. are also present in relatively low numbers as compared to the numbers of pigmented Flavobacterium sp. present. The water at Vinca bridge contains a similar distribution of FANU when compared to 156 St. location with possibly a slight enrichment of the numbers of Acinetobacter sp. at the expense of the Flavobacterium sp. However, the sediment at Vinca bridge shows a complete change when compared to that of 156 St. or the source in that the FANU are comprised of 90% Alcaligenes sp. and 10% Aeromonas sp.

Facultative anaerobes are not present in either the sediment or the water at the source sample site. Members of this group become increasingly prevalent in the sediment at 156 St. and more prevalent on the water and sediment downstream from Edmonton at Vinca bridge. The source is the only site where the sediment bears any similarity to the water in terms of the bacterial genera present.

TABLE 18

CHARACTERIZATION OF LONG CHAIN FATTY ACID NON UTILIZING BACTERIAL ISOLATES FROM SEDIMENT AND WATER SAMPLES.

Sample Date	Sample Location	Sample Type	Family or Genera as a Percentage of the Total F.A.N.U.*						
			Flavo-bacterium	Acine to-bacter	Alcali-genes	Aeromonas	Micro-coccus	Pseudomonas	Cytophaga
3/6/74	156 St.	Water	50.0	12.5	31.2	6.3	-	-	-
3/6/74	156 St.	Sed. I**	70.0	-	20.0	-	-	10.0	-
3/6/74	156 St.	Sed.II***	66.6	-	33.4	-	-	-	-
4/6/74	Vinca bridge	Water	47.1	11.8	41.1	-	-	-	-
4/6/74	Vinca Br.	Sed.+	-	-	90.0	10.0	-	-	-
24/6/74	Source	Water	50	25.0	-	-	-	-	25.0
24/6/74	Source	Sed.++	22.2	27.8	44.4	-	-	-	5.6

* = Long chain fatty acid non utilizer

** = Sediment I (See Table 16)

*** = Sediment II (See Table 16)

\+ = Sediment - Course sand, fine sand, silt, organic material, sulphide odor

++ = Sediment - Large rocks, glacial flour

IX. Salmonella isolation.

The original method of Salmonella sp. retrieval from natural waters as described by Moore(1948) was modified slightly to suit this study for 2 reasons. The swab size was reduced to minimize the entrapment of large debris. Contact time was varied inversely with the flow rate of the aquatic system being sampled. The swab was suspended for half an hour in the sewage effluent and for an hour in the river since the water velocity in both cases was apparently greater than that in the system sampled by Moore. Although there was a very rapid flow rate at the source it was sampled for 4 hours since the bacterial population was low and there was less probability of the presence of Salmonella sp. Generally, Salmonella species were not found in river water. There was 1 exception where Salmonella infantis was isolated at 156 St. on 3/6/74 and at Vinca bridge on the following day(Table 19). Previously, Salmonella alachua had been isolated from the water at Vinca bridge(Coleman et al., 1974). Water from the source sample site yielded no Salmonella sp. by this method. Three positive isolations were obtained out of 8 samples of the sewage effluent. Two different species were isolated from the same sample(29/4/74). It appears that there is a sporadic discharge of Salmonella sp. from the Edmonton domestic sewage treatment plant. This

TABLE 19

SALMONELLA ISOLATIONS AT 156 ST. SEWAGE EFFLUENT AND VINCA BRIDGE SAMPLE SITES

Sample Date	Sample Location	Isolation	Species Isolated
6/9/73	156 St.	Neg	-
	Sewage Effluent	Neg	-
	Vinca Bridge	Neg	-
14/11/73	156 St.	Neg	-
	Sewage Effluent	Pos	S. infantis
	Vinca Bridge	Neg	-
24/1/74	156 St.	Neg	-
	Sewage Effluent	Neg	-
	Vinca Bridge	Neg	-
2/4/74	156 St.	Neg	-
	Sewage Effluent	Neg	-
	Vinca Bridge	Neg	-
22/4/74	156 St.	Neg	-
	Sewage Effluent	Neg	-
	Vinca Bridge	Neg	-
29/4/74	156 St.	Neg	-
	Sewage Effluent	Pos	S. eastbourne S. typhimurium
	Vinca Bridge	Neg	-
21/5/74	156 St.	Neg	-
	Sewage Effluent	Pos	S. orienburg
	Vinca Bridge	Neg	-
15/7/74	156 St.	Neg	-
	Sewage Effluent	Neg	-
	Vinca Bridge	Neg	-
3/6/74	156 St.	Pos	S. infantis
4/6/74	Vinca Bridge	Pos	S. infantis

suggests that either the occurrence of *Salmonella sp.* is not predictable or the sampling method is not entirely effective.

C. CHEMICAL.

I. Extraction techniques and the recovery of lipid material from sewage effluent.

A comparative study was done in order to evaluate various methods of lipid extraction. Three different procedures were used. The method used by the City of Edmonton sewage treatment plant laboratory and involved Soxhlet extraction using n-hexane as solvent. The method of P. Fedorak, of this department, which utilized a Soxhlet extraction technique using Freon TF(1,1,2-trifluoro 1,2,2-trichloroethane) as the solvent. Chloroform-methanol reflux method was used in this study. The application of these methods to the analysis of a sample of Edmonton domestic sewage effluent yielded 22.2 mg/l, 15.3 mg/l and 28.6 mg/l, respectively. Differences between the analytical methods used are probably due to solvent characteristics, that is, difference in their boiling points and dielectric constants. The differences could also lie in the assay used for lipid material. The City of Edmonton and P. Fedorak's methods involved a gravimetric procedure for analysis of the residue

whereas a spectrophotometric method was used in this study. A comparison of the reflux and separatory funnel extraction methods was also carried out. The reflux method was superior in that more lipid material was extracted by the this method as compared to to the simple separatory funnel extraction procedure (Table 20). Table 20 shows that the effect of filtering the domestic sewage effluent with an 0.45u filter is to remove about 11% of the lipid material as determined by the dichromate assay. Both cell bound lipids and those bound by hydrophobic interactions to particulate material are most likely removed by the filtration step. Future analysis did not involve filtration since the total lipid profile was sought.

On the basis of these results acidified samples were assayed for lipid material and long chain fatty acids using chloroform-methanol solvent and the reflux method.

The maximum amount of lipid material was released after 3 hours of reflux (Figure 1) and showed no subsequent change up to 6 hours. In order to facilitate the handling of a large number of samples a 4 hour reflux time was used in all lipid analyses.

TABLE 20

COMPARISON OF EFFICIENCY OF LIPID EXTRACTION FROM DOMESTIC SEWAGE EFFLUENT USING THE REFLUX AND THE SEPARATORY FUNNEL TECHNIQUES

Procedure	mg/l Lipid Material* Non-Filtered	Filtered**
Separatory Funnel Extraction	5.04	4.92
Reflux Extraction	6.80	6.08

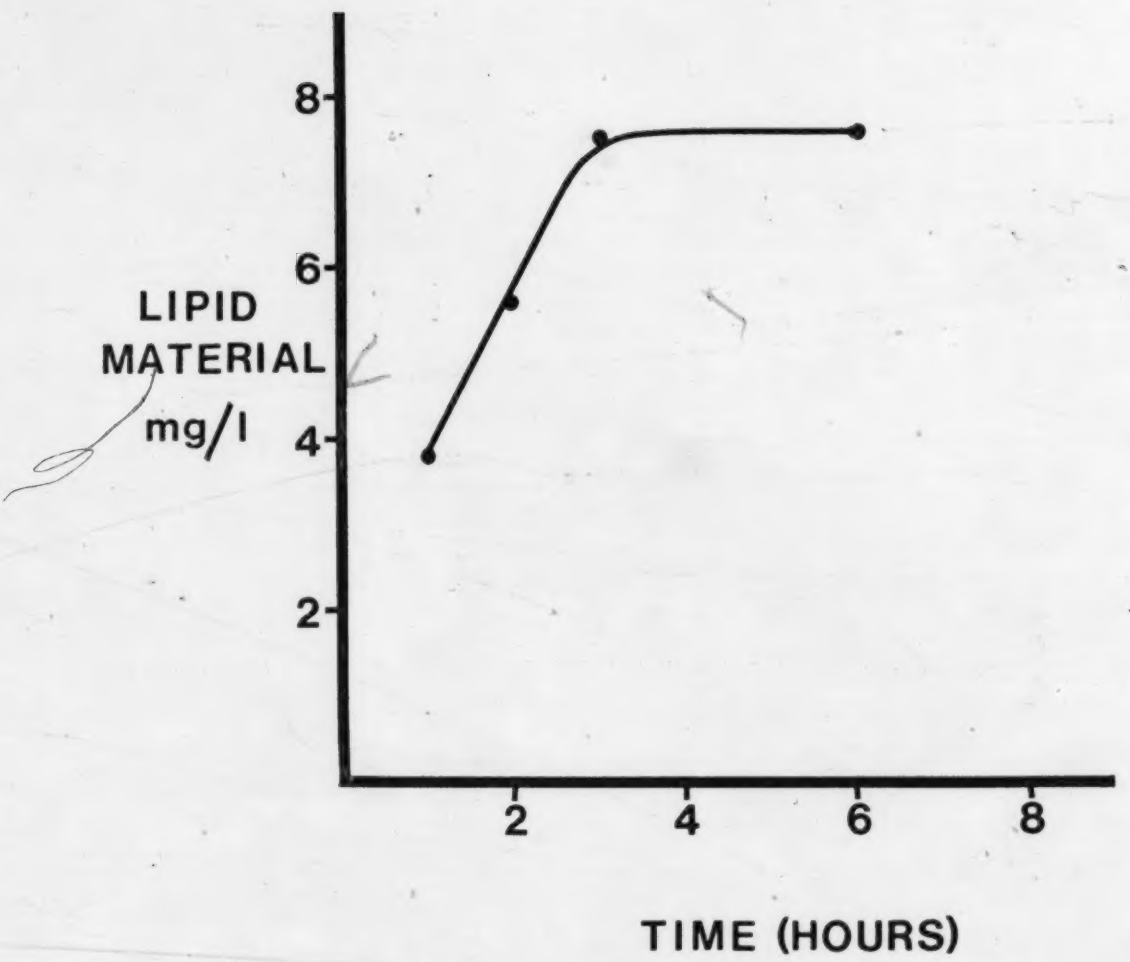
* = Lipid material determined by sodium dichromate assay

** = 0.45μ Filter (Millipore)

FIGURE I

EFFECT OF REFLUX TIME UPON THE RELEASE OF LIPID MATERIAL FROM DOMESTIC SEWAGE EFFLUENT

The sample was refluxed at 61 degrees in four volumes of 2:1 chloroform methanol. The lipid material was that material dissolved in the chloroform layer and was quantitated by the dichromate assay.


8
6
4
2
LIPID
MATERIAL
mg/l
2
4
6
8
TIME (HOURS)

II. Analysis of North Saskatchewan river water and sewage effluent for lipid material and long chain fatty acids throughout the sample year.

The total lipids(Tables 21,22 and 23) vary over the sample year to such an extent that no inference may be drawn as to the significance of this analysis. The definition of lipid material involves the oxidation of any chemical species that are present in the river and soluble in the chloroform-methanol solvent system. Lipid material may include pigments, sterols, vitamins as well as other long chain fatty acids such as the cyclopropane and B-hydroxy fatty acids. Therefore, the assay, as defined, is of little use with respect to long chain fatty acids. As best as can be estimated in view of this assay limitations it appears that 5-28% of the total lipid material may be long chain fatty acid. The exception was the period when pulse loading occurred where a significantly higher portion of the lipid material was fatty acids(39%).

The total of the long chain fatty acids present at the 156 St. site show little change in concentration from the amount found at the Vinca bridge sample site(Tables 21 and 23). This indicates that the sewage effluent has little effect on the fatty acid content of the river water. The fatty acid content of the sewage effluent is shown in Tables 22 and 25. It was found

TABLE 21

TOTAL LIPIDS AND TOTAL LONG CHAIN FATTY ACID ANALYSIS OF WATER FROM 156 ST. SAMPLE SITE

Sample Date	µg/l Total Lipids*	Total L.C.F.A.**	Ratio L.C.F.A/Lipid
6/9/73	1,400	194	0.139
14/11/73	4,600	224	0.048
24/1/74	2,700	307	0.115
2/4/74	400	80	0.199
22/4/74	2,000	450	0.226
29/4/74	1.600	162	0.103
21/5/74	3,600	513	0.143
15/7/74	1,300	249	0.190

* = Analysis by dichromate assay

** = Long chain fatty acid, analyzed by G.L.C.

TABLE 22

TOTAL LIPIDS AND LONG CHAIN FATTY ACID ANALYSIS OF WATER FROM SEWAGE EFFLUENT SAMPLE SITE

Sample Date	µg/l Total Lipids*	Total L.C.F.A.**	Ratio L.C.F.A./Lipid
6/9/73	12,900	1077	0.083
14/11/73	10,300	811	0.079
24/1/74	7,900	3085	0.391
2/4/74	3,300	540	0.167
22/4/74	6,400	1695	0.174
29/4/74	3,400	1092	0.277
21/5/74	14,500	660	0.046
15/7/74	2,700	482	0.177

* = Analysis by dichromate assay

** = Long chain fatty acid, analyzed by G.L.C.

TABLE 23

TOTAL LIPIDS AND LONG CHAIN FATTY ACID ANALYSIS OF WATER FROM VINCA BRIDGE SAMPLE SITE

Sample Date	µg/l Total Lipids *	Total L.C.F.A. **	Ratio LCFA/Lipid
6/9/73	1600	152	0.097
14/11/73	1700	319	0.191
24/1/74	2100	166	0.082
2/4/74	1300	215	0.173
22/4/74	2200	389	0.179
29/4/74	1200	475	0.396
21/5/74	2100	147	0.071
15/7/74	1500	271	0.183

* = Analysis by dichromate assay

** = Long chain fatty acids; analyzed by G.L.C.

that generally the domestic sewage effluent instantaneous dilution factor by the North Saskatchewan river was 0.01 (see Table 31). Therefore, the amount of sewage effluent derived long chain fatty acid found at Vinca bridge will be minimal since the dilution factor is high. If a calculation is made from the data in Table 31 the effluent derived fatty acids at Vinca bridge will be at most 0.031 mg/l or at least 0.005 mg/l representing 21% and 1.1% of the total found, respectively. These percentages being the extreme cases of high spring sewage loading rates on low winter river flow and vice versa. The long chain fatty acid composition of the chloroform:methanol extractable material as revealed by G.L.C. analysis for the sample sites are shown in Tables 24, 25 and 26. The sewage effluent contains higher concentrations of unsaturated fatty acids compared to those found in the river water samples. The fact that the river water contains lower concentrations of unsaturated fatty acids suggests that the effect of the sewage effluent on the river is minimal. Bacterial cells appear not to contribute significantly to the total long chain fatty acid concentration of the river. This was rationalized from the fact that dried bacterial cells contain, at most, about 10-15% lipid material or about 15ug/mg dry weight (Carpenter, 1967). A mg of dry bacterial cells is approximately equivalent to about 10^{10} cells, therefore

TABLE 24

LONG CHAIN FATTY ACID ANALYSIS OF 156 ST. SAMPLE SITE RIVER WATER

Sample Date	Long Chain Fatty Acid - µg/l							
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
6/9/73	8	17	26	24	11	0	0	0
14/11/73	0	22	47	28	89	37	0	0
24/1/73	0	28	133	0	107	39	0	0
2/4/73	0	10	27	14	15	14	0	0
22/4/73	13	35	54	59	220	69	0	0
29/4/73	0	20	35	33	78	0	0	0
21/5/74	0	35	69	45	181	72	111	0

TABLE 25

LONG CHAIN FATTY ACID ANALYSIS OF WATER FROM SEWAGE EFFLUENT SAMPLE SITE

Sample Date	Long Chain Fatty Acid ug/l							
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
6/9/73	36	105	230	123	384	199	-	-
14/11/73	31	91	241	187	260	-	-	-
24/1/74	7	138	426	207	772	705	480	349
2/4/74	9	42	153	44	203	88	-	-
22/4/74	27	133	268	170	842	255	-	-
29/4/74	-	103	259	102	495	133	-	-
21/5/74	57	94	115	77	103	215	-	-
15/7/74	122	125	150	72	13	-	-	-

TABLE 26

LONG CHAIN FATTY ACID ANALYSIS OF WATER FROM VINCA BRIDGE SAMPLE SITE

Sample Site	Long Chain Fatty Acid µg/l							
	C12:0	C14:0	C:16:0	C16:1	C18:0	C18:1	C18:2	C18:3
6/9/73	1	16	31	13	90	-	-	-
14/11/73	-	15	35	27	205	37	-	-
24/1/74	6	17	28	25	91	-	-	-
2/4/74	7	19	44	80	36	-	-	-
22/4/74	2	30	67	47	166	77	-	-
29/4/74	26	44	83	54	217	51	-	-
21/5/74	-	8	27	15	24	29	25	20
15/7/74	47	34	43	46	99	3	-	-

1.5 pg of lipid material will be contained in about 10^3 viable bacterial cells. The highest river count at 156 St. was 4.1×10^5 cells/ml or 4.1×10^8 cells/l found on 22/4/74 (Tables 6,7 and 8). This corresponds to approximately 450 ug/l of total lipid derived from viable bacterial cells. Assuming that the bacterial lipid content was entirely long chain fatty acid in nature then the bacterial contribution would be 0.615 ug/l (only 0.14% of the total). A similar calculation for the sewage effluent at the same sample time yielded 1.02 ug/l of bacterially derived fatty acids. This is less 0.1% of the total (1.695 mg/l) present in the effluent at that time. This is of course assuming that lipid material is entirely long chain fatty acid. There may be a contribution to the long chain fatty acid concentration by dead bacterial cells.

Dodecanoic acid (12:0) appears to be a very minor fraction of the total long chain fatty acid present in water at the river sites. However, the sewage effluent contains considerably more on a per volume basis with the exception of the samples taken on 29/4/74 where it was not detected.

Tetradecanoic (14:0) acid, while always being present, is also a minor component of the total fatty acids present in the river water. The sewage effluent however always contains this fatty acid in a much higher

concentration.

Hexadecanoic acid(16:0) is one of the most prevalent long chain fatty acids in nature, apparently contained in most life forms. The actual concentration of this acid at both river sites is remarkably constant throughout the sample year with typical levels ranging from about 0.02 mg/l to 0.08 mg/l. The exception to this was the very high level found on 21/1/74 at 156 St. The fall and winter levels are typically around 0.02 mg/l whereas the late spring and summer values range between 0.035 mg/l to 0.083 mg/l. The sudden increase occurs at breakup(22/4/74) with the high level being maintained until the last sample date(15/7/74).

Cis-9-hexadecenoic acid(16:1) is usually present in river water throughout the sample year with the exception at 156 St. of the sample taken on 21/1/74. The level found was approximately 60-70% of that of the hexadecanoic acid concentration. The sewage effluent contained much less cis-9-hexadecenoic acid relative to hexadecanoic acid, usually being in the order of 50% or less.

Both the sewage effluent and the river contain significantly higher levels of octadecanoic acid(18:0) when compared to that of hexadecanoic acid, the differences being from 2 to 4 times as much. The only

exception to this occurred 2/4/74 where at both 156 St. and Vinca bridge sample sites the ratio is reversed, that is, the levels of hexadecanoic acid exceed that of octadecanoic acid. The sewage effluent showed has no such difference, maintaining a higher concentration of octadecanoic acid in all samples.

Cis-9-octadecenoic acid(18:1) concentration varied in all samples taken throughout the year. The 156 St. site, for example, did not contain any 18:1 in the water during the summer months whereas it was found in the winter. Vinca bridge, on the other hand, contains this fatty acid only after breakup. This suggests that during the winter the bacterial population in the river downstream from Edmonton may be hydrogenating the double bond to yield octadecanoic acid or is utilizing this fatty acid(Table 26). Presence of cis-9-octadecenoic acid downstream at Vinca bridge on 11/11/73 is explained by the fact that the sediment was disturbed at this time due to ice flow conditions. It is suggested that this acid is coming from the sediment since the levels at Vinca bridge and 156 St. are the same.

Cis,cis-9,12-octadecdienoic acid(18:2) was only present at both river sites on 21/5/74 and at no other time. This may indicate that if any of this acid is introduced, which it should be since its origins are from plant material, it was hydrogenated to eliminate

either or both double bonds. This is known to occur in the rumen(Dawson and Kemp, 1970). The sewage effluent generally does not contain any of this acid with the exception of the sample taken on 24/1/74 when a pulse loading situation occurred. Bulking, the result of pulse loading, was followed by poor treatment thus giving high bacterial counts(Table 7). During this time, high concentrations of long chain fatty acids tending toward the unsaturated 18 carbon variety were observed in the sewage effluent(Table 25).

Cis,cis,cis-9,12,15-octadecatrienoic acid(18:3) was found in the sewage effluent during bulking but never in the river with the exception of Vinca bridge on 21/5/74. This latter observation when compared to the findings of the samples throughout the year indicates this observation at Vinca bridge is suspect and possibly due to sample contamination.

III. Analysis of physical and chemical data from the North Saskatchewan river water and sediment and sewage effluent throughout the sample year.

The sample dry weight varies little between 156 St. and Vinca bridge sample sites, in fact there appears to be a slight decrease in the amount present at the latter site. This is probably due to a decrease in velocity at the Vinca bridge river site. The channel width at Vinca

bridge increases but the discharge rate does not change significantly between 156 St. and Vinca bridge. This allows greater sedimentation(Tables 27 and 30). During breakup on 22/4/74 the sample dry weight increases to approximately twice the value found for the rest of the year. The dry weight at the source water sample site was a high value of 1450 mg/l. The sewage effluent(Table 28) sample dry weight varies with no apparent pattern. The value on 2/4/74 is high, presumably due to street runoff water but is not not much higher than that of the sample taken on 15/7/74(Table 28).

Inorganic carbon concentrations of water at the local sample sites varies too excessivly. Therefore few conclusions can be drawn from these data. There is 1 possible exception, that being when the sewage effluent flow rate is high on 22/4/74(Tables 28 and 31) the inorganic carbon concentration is low, possibly as a result of dilution. The sample taken at the source has a high value for inorganic carbon(Table 33) indicating that much of the dry sample weight is carbonate in nature(glacial flour).

Organic carbon values vary more widely than do the inorganic carbon values. A slight trend, however, was indicated during the winter months since the concentration does drop slightly during this time. The

TABLE 27

PHYSICAL AND CHEMICAL DATA OF WATER FROM 156 STREET SAMPLE SITE

mg/l

Sample Date	Dry Weight	Inorganic Carbon	Organic Carbon	Total Carbon	Ammonia Nitrogen	Organic Nitrogen	Total Nitrogen
6/9/73	632.8	27.5	8.5	36.0	0.04	0.3	0.34
14/11/73	426.7	31.0	13.0	44.0	ND	0.74	0.74
24/1/74	367.0	15.5	4.0	19.5	ND	0.15	0.15
2/4/74	360.0	13.5	0.5	14.0	ND	0.19	0.19
22/4/74	1423.3	11.5	34.5	46.0	ND	2.7	2.7
29/4/74	730.0	47.5	34.0	81.5	ND	1.3	1.3
21/5/74	440.0	16.5	16.0	32.5	ND	0.48	0.48
15/7/74	410.0	23.5	28.0	51.5	ND	0.62	0.62

ND - None detected

TABLE 28

PHYSICAL AND CHEMICAL DATA OF WATER FROM SEWAGE EFFLUENT SAMPLE SITE

mg/l

Sample Date	Dry Weight	Inorganic Weight	Organic Carbon	Total Carbon	Ammonia Nitrogen	Organic Nitrogen	Total Nitrogen
6/9/73	910.6	28.0	23.7	51.7	20.8	2.5	23.3
14/11/73	473.3	38.0	30.0	68.0	21.0	2.8	23.8
24/1/74	497.0	23.0	25.5	48.5	20.7	2.1	22.8
2/4/74	491.0	25.0	16.0	41.0	1.3	4.4	5.7
22/4/74	1300.0	16.0	22.5	38.5	1.9	4.2	6.1
29/4/74	936.7	22.0	17.5	39.5	3.0	2.4	5.4
21/5/74	700.0	32.0	19.0	51.0	1.2	3.1	4.3
15/7/74	1286.7	42.5	22.5	65.0	7.9	2.5	10.4

TABLE 29

PHYSICAL AND CHEMICAL DATA OF WATER FROM VINCA BRIDGE SAMPLE SITE

mg/l

Sample Date	Dry Weight	Inorganic Carbon	Organic Carbon	Total Carbon	Ammonia Nitrogen	Organic Nitrogen	Total Nitrogen
6/9/73	556.1	27.0	6.5	33.5	0.21	0.33	0.54
14/11/73	293.3	32.0	12.0	44.0	ND	0.32	0.32
24/1/74	310.0	19.5	8.5	28.0	ND	0.11	0.11
2/4/74	307.0	8.0	3.0	11.0	ND	0.55	0.55
22/4/74	910.0	11.5	35.0	46.5	ND	2.2	2.2
29/4/74	686.7	13.0	20.5	33.5	ND	1.7	1.7
21/5/74	486.7	16.5	34.0	50.5	ND	0.85	0.85
15/7/74	650.0	19.5	24.0	43.5	ND	1.2	1.2

ND - None detected

sewage effluent data reveals a more constant picture as might be expected. There is no indication that the total carbon concentration increases within the river as it passes through Edmonton , similar to the observation of no significant change in concentration of the long chain fatty acids. Organic carbon values at the source sample site present a more disconcerting picture in that the level is 22.0 mg/l, a higher value than was observed for the 156 St. sample site during the summer.

The ammonia nitrogen data does not reveal any trend. One might expect an occasional appearence at Vinca bridge(Table 29) since ammonia is found in the domestic sewage effluent(Table 28). Ammonia nitrogen was not detected at 156 St. except in the sample taken on 14/9/73 where it was found to be 0.04 mg/l. This value is approaching the level of sensitivity of the method or possibly may be due to sample contamination(Table 27). Domestic sewage effluent shows as expected high levels present with the range being from 1.2 mg/l to 21.0 mg/l. Ammonia is very likely to be taken up rapidly by the indigenous river microflora upon contact, oxidised to nitrate or absorbed to negatively charged colloids(clay). The source sample, as expected, showed no detectable ammonia nitrogen(Table 33).

The concentration of organic carbon present in the

water at the 156 St. site tends to be low, less than 0.7 mg/l throughout the year. The exception occurred on 14/11/73 when the sediment was disturbed and during breakup, 22/4/74 and 29/4/74. The content at Vinca bridge varies little compared to 156 St. This indicates that the City of Edmonton contributes little nitrogen to the river or that the increased microbial numbers due to the effect of the sewage effluent may metabolize the organic nitrogen before the water reaches Vinca bridge. The sewage effluent organic nitrogen level is fairly constant throughout the sample year, a range of between 2.1 mg/l to 4.4 mg/l, with the high levels occurring during spring thaw. Organic nitrogen levels at the source sample site are very low, 0.09 mg/l, a level approaching the sensitivity of the analytical method.

The pH of the surface water at the 156 St. sample site changes very little throughout the year. The lowest value, pH 7.8, occurred at breakup on 22/4/74, and the highest value, pH 8.3, was found in samples obtained on 6/9/73 and 24/1/74 (Table 30). The low value for the breakup period possibly is the result of the release of acidic organic components from the sediment. Generally, the pH at Vinca bridge is lower than that of 156 St. indicating a possible influence of the City's domestic sewage effluent on the river as was found previously (Coleman et al., 1974). The lowering of the

TABLE 30

PHYSICAL DATA FROM WATER AT 156 ST. SAMPLE SITE

Sample Date	pH	Temp.
6/9/73	8.3	15
14/11/73	8.1	1
24/1/74	8.3	< 1
2/4/74	8.0	1
22/4/74	7.8	1
29/4/74	8.0	5
21/5/74	8.1	9
15/7/74	8.2	16

TABLE 31

PHYSICAL DATA FROM WATER AT SEWAGE EFFLUENT SAMPLE SITE

Sample Date	pH	Temp.	Litres/day x 10^6 River Discharge	Litres/day x 10^6 Sewage Discharge	Sewage Dilution
6/9/73	7.5	17	18626.8	185.6	0.010
14/11/73	7.4	17	11381.7	173.6	0.015
24/1/74	7.1	15	16032.3	199.3	0.012
2/4/74	7.9	14	10182.3	263.6	0.026
22/4/74	7.9	15	82232.0	274.9	0.003
29/4/74	7.8	17	57275.7	188.0	0.003
21/5/74	7.9	15	41892.0	198.1	0.005
15/7/74	7.4	18	42382.1	247.6	0.006

TABLE 32

PHYSICAL DATA FROM WATER AT VINCA BRIDGE SAMPLE SITE

Sample Date	pH	Temp.
6/9/73	8.4	17
14/11/73	7.9	1
24/1/74	8.0	1
2/4/74	7.9	1
22/4/74	7.7	3
29/4/74	8.0	8
21/5/74	8.0	11
15/7/74	7.7	18

TABLE 33

PHYSICAL AND CHEMICAL DATA OF WATER AT THE SOURCE

SAMPLE SITE ON JUNE 24, 1974

mg/l						
Dry Weight	Inorganic Carbon	Organic Carbon	Total Carbon	Ammonia Nitrogen	Organic Nitrogen	Total Nitrogen
1450.0	47.5	22.0	69.5	ND	0.09	0.09

Temp.	pH
2	8.7

ND - None detected

pH at breakup is also seen at Vinca bridge(Table 32). The sewage effluent exhibited a fairly constant pH being in the range of 7.4 to 7.9 with the exception of 24/1/74 where pulse loading was in progress and the value was 7.1. These changes correlate well with levels of long chain fatty acids found at this time(Tables 22 and 31).

The water temperature during the summer at the 156 St. and Vinca bridge river sites appear to be approximately equal to those found in previous work(Coleman et al., 1974). A high value of 18°C on 15/7/74 was found in this study compared to a high at Vinca bridge of 17°C in July 1972. The temperatures of all other river samples varied from close to zero under ice conditions up to the high of 18°C during the summer period as previously duscussed. The temperature does increase rapidly from about zero under ice to 5°C at spring breakup 1 week later and to 9°C within a further 3 weeks. The sewage effluent varies only 4°C over the sample year. The lowest reading of 14°C occurred during spring thaw on 2/4/74 and a high of 18°C occurred on 15/7/74. The low temperature would be due to the high volumes on melt water being introduced to the river system. The low temperature(2°C) found at the source agrees with previous findings(Coleman et al., 1974).

River discharge varies from a winter low of 10182.3x10^6 litres/day on 2/4/74 to a spring high at

breakup of 82242.0x10⁶ litres/day occurring on 22/4/74. The discharge of the sewage effluent is fairly constant, the high flow rate being 274.9x10⁶ litres/day for 22/4/74 and the low rate being 173.6x10⁶ litres/day on 11/11/73. This represents only a 31% difference(Table 31). The instantaneous sewage dilution rate at confluence with the river varies from a high at breakup of 0.003 to a low just prior to breakup of 0.026. This is a 10-fold change in the rate of dilution. Over the late spring to summer period the dilution rate falls to a summer low of 0.006 and drops to a lower value in the fall. If the sewage effluent was contributing significantly to the river in any manner then a change in parameters should be seen at Vinca bridge when the effluent is diluted to a lesser extent. This is not the case(Table 12). To further add to this arguement, the carbon and nitrogen data(Table 11) do not show a significant increase at Vinca bridge during low dilution periods.

IV. Analysis of sediment and water for long chain fatty acids.

On a volume basis sediment long chain fatty acids show a marked increase in concentration of those present in the water as compared to those present in the water. Large increases in the amounts of di- and triunsaturated

fatty acids are present in the sediment as compared to those present in the sediment, (Tables 34 and 35), with the amounts of all fatty acids being at least 4 times as abundant in the sediment. The SED II sediment from 156 St. was used as the typical sediment at this location since it was grossly similar to the sediment found at Vinca bridge. Sed I at 156 St. had a heavy growth of epiphytic algae whereas no algae were observed on the sediment at Vinca bridge. The sediment at Vinca bridge shows a 10-fold increase in the total long chain fatty acid concentration as compared to that of SED II from 156 St. Although the level found in the water sampled at this time at the 2 locations is very nearly the same. The levels of fatty acids present in either the water or the sediment at the source sample site are very low. There is no evidence for the presence of unsaturated fatty acids at this location. The total lipid values found in the water at the 156 St. and Vinca bridge sites show similar levels as compared to those found at other times (Tables 24,26,21 and 23) whereas the values in the sediment show a wide variation, 56.0 mg/l for SED II at 156 St. and 27.2 mg/l at Vinca bridge. The total fatty acids present in the same samples show an inverse relationship. The fraction of the total lipid that are long chain fatty acids are just as variable in the sediment as they are in the water.

TABLE 34

COMPARISON OF LONG CHAIN FATTY ACID PROFILES IN SEDIMENT WITH THOSE IN WATER AT 156 STREET, VINCA BRIDGE AND THE SOURCE

Sample Date	Sample Location	Sample Type	Fatty Acid µg/l							
			C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
3/6/74	156 St.	Water	18	19	62	24	33	18	3	-
3/6/74	156 St.	Sed.I*	22	187	365	426	407	338	327	988
3/6/74	156 St.	Sed.II**	-	5	178	115	46	41	-	58
4/6/74	Vinca Br.	Water	12	59	21	60	13	-	-	-
4/6/74	Vinca Br.	Sed.***	4	196	479	313	244	248	21	260
25/6/74	Source	Water	-	-	3	-	4	-	-	-
25/6/74	Source	Sed.+	-	-	150	-	138	-	-	-

* = Sediment I - Sediment consisting of small rocks, gravel and silt

** = Sediment II- Sediment consisting of coarse sand and a little silt

*** = Sediment consisting of fine sand, silt and organic material

\+ = Sediment consisting of larger rocks, small rocks and glacial flour

TABLE 35

COMPARISON OF TOTAL LONG CHAIN FATTY ACIDS AND TOTAL LIPIDS IN WATER WITH THOSE IN SEDIMENTS AT 156 STREET VINCA BRIDGE AND THE SOURCE SITES

			µg/l		
Sample Date	Sample Location	Sample Type	Total LCFA*	Total Lipid	Ratio LCFA*/Lipid
3/6/74	156 St.	Water	176	640	0.275
3/6/74	156 St	Sed.I**	3081	15680	0.196
3.6.74	156 St.	Sed.II**	444	56000	0.008
4/6/74	Vinca Bridge	Water	165	780	0.212
4/6/74	Vinca Bridge	Sed.***	1765	27200	0.065
25/6/74	Source	Water	7	80	0.088
25/6/74	Source	Sed.+	288	2720	0.106

*Long chain fatty acids

**Sed.I = Sediment consisting of small rocks, gravel and silt

Sed.II = Sediment consisting of coarse sand and a little silt

***Sed. = Sediment consisting of fine sand, silt and organic material.

+ = Sediment consisting of large rocks, small rocks and glacial flour

D. STATISTICAL ANALYSIS OF EXPERIMENTAL DATA.

The simple correlation coefficient as previously mentioned measures or ascribes a number to the relationship between 2 measurable parameters as to the°C of relatedness. The magnitude of the number being directly proportional. Therefore, as the number, designated r, approaches 1 the relatedness increases. In order that r contain any meaning, its value is compared to a table of r values that are dependent upon the°C of freedcm of the sample size.

In Table 36 the significant r values are presented between independent variables grouped together from all local sample sites throughout the sample year. The critical value for r at p=0.05 is 0.404. Using this value, there is no correlation between total count and the FAU but there is with pigmented count. It is of interest tc note that the numbers of FAU in the fall and winter samplings represent approximately 1% of the total count whereas in the spring and summer samplings they represent 10% of the total ccunt(Tables 7 and 8). The pigmented count represents 30-50% of the total count. As per the total count, the pigmented count shows no correlation with the FAU which bears out the observation that the classification of the FAU show them to be non-pigmented species(Tables 10,11 and 12). This is an interesting situation which however does not hold when

TABLE 36

SIMPLE CORRELATION COEFFICIENT (r) BETWEEN SOME MEASURED VARIABLES OF WATER FROM COMBINED DATA OF SAMPLE SITES 156 STREET SEWAGE EFFLUENT AND VINCA BRIDGE

	r-values *		
	Dependent Variable		
Variable	Total Count	Pigmented Count	F.A.U.** Count
Total Count	1,000	0.980	0.383
Pigmented Count	0.980	1.000	0.398
F.A.U. Count	0.383	0.398	1.000
Total Lipid	0.446	0 516	0.725
Dodecanoic Acid	0.048	0.086	0.574
Tetradecanoic Acid	0.585	0.633	0.525
Hexadecanic Acid	0.742	0.773	0.285
Cis-9-Hexadecenoic Acid	0.665	0.695	0.295
Octadecanoic Acid	0.613	0.644	0.062
Cis-9-Octadecenoic Acid	0.937	0.946	0.395
Cis,cis-9,12-Octadec-dinenoic Acid	0.931	0.886	0.153
Cis,cis,cis-9,12,15-Octadecatrienoic Acid	0.963	0.920	0.175
Total Fatty Acid	0.879	0.891	0.280
Dry Weight	-0.035	0.029	0.181
Ammonia Nitrogen	0.636	0.711	0.269
Organic Nitrogen	0.248	0.283	0.383
Total Nitrogen	0.257	0.434	0.240
pH	-0.619	-0.638	-0.738
River Discharge	-0.124	0.110	0.032
% Alcaligenes of Total F.A.U.	0.689	0.673	0.464

* At p=0.05, Critical value 0.404

** FAU = Long chain fatty acid utilizer

the individual sites are analysed separately.

The numbers of FAU are positively correlated with the total lipids, with the concentrations of both dodecanoic acid and tetradecanoic acid and the fraction of the FAU that are of the genus Alcaligenes. The numbers of FAU are negatively correlated with the pH. This negative correlation may be spurious since the FAU count increases in the sewage effluent(Table 7) where the pH is generally lower(Table 31). The positive correlation of the FAU with both the concentrations of dodecanoic acid and tetradecanoic acid appears logical since the FAU counts are stimulated or are higher in the sewage effluent where the long chain fatty acids are found. The somewhat unexpected correlation of both the total count and the pigmented count occurs with certain long chain fatty acids. This may result from the fact that the numbers of many microbial types were observed to increase with an increase in long chain fatty acid concentration in the sewage effluent.

The concentration of ammonia nitrogen shows a positive significant correlation with both total count and the pigmented count. However, the organic nitrogen does not to correlate significanctly with the total and pigmented counts. This indicates the possible inaccessibility of complex nitrogen compounds to the bacterial cell.

The statistical analysis of the results obtained from the samples taken at 156 St. site gave a slightly diiferent picture(Table 37). There is a correlation of the FAU with the total ccunt and somewhat dissappointingly with the pigmented count. This latter correlation was not expected since no pigmented bacterial isolates were capable of growth on the TYA+FA agar plate(Tables 13,14 and 15). This spurious correlation may be explained from the point of view that there is significant correlation of the pigmented count with the total ccunt of which the FAU are a fractional portion. Therefore, since the FAU count and the pigmented count are portions of the total count, then the spurious correlation of the pigmented count with the FAU count may cccur.

A significant correlation was found between the sample dry weight and numbers of all the bacterial groups(Table 37), suggesting that a close association or attachment exists between the 3 grcups and the suspended particulate material.

The content of organic nitrogen and subsequently total nitrcgen, yields a positive significant correlation with all 3 microbial groups. This indicates that there is possibly an association of the microbial cells with the nitrogen source which may be an important

TABLE 37

SIMPLE CORRELATION COEFFLUENT (r) BETWEEN SOME MEASURED VARIABLES OF WATER FROM 156 STREET SAMPLE SITE

	r-values*		
	Dependent Variable		
Variable	Total Count	Pigmented Count	F.A.U*** Count
Total Count	1.000	0.999	0.996
Pigmented Count	0.999	1.000	0.995
F.A.U. Count	0.996	0.995	1.000
Total Lipid	-0.121	-0.126	-0.099
Dodecanoic Acid	0.671	0.666	0.674
Tetradecanoic Acid	0.360	0.354	0.365
Hexadecanoic Acid	-0.066	-0.071	-0.040
Cis-9-Hexadecenoic Acid	0.699	0.699	0.684
Octadecanoic Acid	0.598	0.596	0.620
Cis-9-Octadecenoic Acid	0.431	0.427	0.474
Cis,cis-9,12-Octadecadienoic Acid	-0.173	-0.175	-0.162
Cis,cis,cis-9,12,15-Octadecatrienoic Acid **	-	-	-
Total Fatty Acid	0.446	0.441	0.472
Dry Weight	0.939	0.941	0.934
Ammonia Nitrogen	-0.198	-0.198	-0.181
Organic Nitrogen	0.961	0.962	0.940
Total Nitrogen	0.961	0.962	0.940
pH	-0.762	-0.765	-0.743
River Discharge	0.848	0.850	0.811
% Alcaligenes of Total F.A.U.	0.868	0.867	0.893

*At p=0.05 Critical value 0.707

**cis,cis,cis-9,12,15-Octadecatrienoic acid not detected at this site.

***FAU = Long chain fatty acid utilizer

portion of the dry sample weight. Another possibility is that the presence of organic nitrogenous material tends to enhance the numbers of the 3 microbial groups at this site. At this time mention should be made, although not reported in the tables, that there was significant correlation between sample dry weight and organic nitrogen ($r=0.896$ at $p=0.05$ with the critical value being 0.707).

The pH correlates inversely with numbers of all microbial groups. That is, it was found that as the bacterial counts increased at breakup there was an associated decrease in the pH (Tables 6 and 18). These may be spurious since no inference can be drawn between the decrease in numbers of the bacteria and the increasing pH values at this sample site (156 St.). To complement this finding, a positive correlation was found between the river discharge and the numbers of the 3 microbial groups. A negative correlation existed between the river discharge and pH ($r=-0.662$ at $p=0.05$, critical value was 0.707).

Since there is a significant correlation between the total count and the FAU count at the 156 St. site there appears to be a microbial population that can utilize many substrates. As stated before the pigmented count is part of the total count. Although they are unable to grow on or use fatty acids they do correlate

significantly with the FAU. There is also a significant correlation indicating the dependence of all microbial groups observed on the presence of organic nitrogenous material.

The data from the sewage effluent presents a much different situation than what was observed at the 156 St. site. For example the FAU count is found not to be a significant part or correlated with either the total count or the pigmented count present in the sewage effluent(Table 38). Again, as at the 156 St. site, there is a positive correlation between the pigmented count and the total count. The concentrations of the individual long chain fatty acids and the total fatty acids correlate positively with the total count and with the pigmented count but are not correlated with the FAU count. This may further indicate that the FAU count is not a unique microbial entity, but rather, long chain fatty acid oxidation appears to be secondary to other microbial metabolic capabilities. The variation found in the fraction of genus Alcaligenes present appears not to be due to the FAU count, whereas they appear to be a significant part of the total count(Tables 11 and 38).

The data in Table 39 shows that water samples from the Vinca bridge site are different from those obtained at 156 St.(Table 37). For example the FAU count does not correlate significantly with the pigmented count,

TABLE 38

SIMPLE CORRELATION COEFFICIENT (r) BETWEEN SOME MEASURED VARIABLES OF WATER FROM SEWAGE EFFLUENT SAMPE SITE

Variable	r - values* Dependent Variable		
	Total Count	Pigmented Count	F.A.U.** Count
Total Count	1.000	0.976	0.166
Pigmented Count	0.976	1.000	0.158
F.A.U. Count	0.166	0.158	1.000
Total Lipid	0.183	0.258	0.600
Dodecanoic Acid	-0.283	-0.276	0.456
Tetradecanoic Acid	0.453	0.479	0.099
Hexadecanoic Acid	0.768	0.775	-0.398
Cis,9-Hexadecenoic Acid	0.565	0.563	-0.283
Octadecanoic Acid	0.482	0.497	-0.425
Cis,9,Octadecenoic Acid	0.930	0.927	0.136
Cis,cis-9,12-Octadecadinenoic Acid	0.984	0.947	0.023
Cis,cis,cis-9,12,15-Octadecatrienoic Acid	0.984	0.947	0.023
Total Fatty Acid	0.874	0.868	-0.146
Dry Weight	-0.552	-0.509	-0.164
Ammonia Nitrogen	0.489	0.577	-0.156
Organic Nitrogen	-0.471	-0.528	-0.218
Total Nitrogen	0.046	0.253	-0.021
pH	-0.579	-0.558	-0.769
River Discharge	-0.321	-0.343	0.015
% Alcaligenes of Total F.A.U.	0.831	0.814	0.489

*At p=0.05 Critical value 0.707

**FAU = Long chain fatty acid utilizer

TABLE 39

SIMPLE CORRELATION COEFFICIENT (r) BETWEEN SOME MEASURED VARIABLES OF WATER FROM VINCA BRIDGE SAMPLE SITE.

Variable	r-values * Dependent Variable: Total Count	Pigmented Count	F.A.U** Count
Total Count	1.000	0.941	0.804
Pigmented Count	0.941	1.000	0.562
F.A.U. Count	0.804	0.562	1.000
Total Lipid	0.385	0.149	0.674
Dodecanoic Acid	0.105	0.285	-0.113
Tetradecanoic Acid	0.692	0.824	0.329
Hehadecanoic Acid	0.868	0.957	0.462
Cis-9-Hexadecenoic Acid	0.711	0.823	0.372
Octadecanoic Acid	0.553	0.623	0.249
Cis-9-Octadecenoic Acid	0.760	0.712	0.553
Cis,cis-9,12-Octadecadinenoic Acid	-0.192	-0.184	-0.210
Cis,cis,cis-9,12,15-Octadecatrienoic Acid	-0.192	-0.184	-0.210
Total Fatty Acid	-0.778	0.876	0.378
Dry Weight	0.853	0.752	0.810
Ammonia Nitrogen	-0.103	-0.257	0.176
Organic Nitrogen	0.921	0.898	0.713
Total Nitrogen	0.936	0.896	0.751
pH	-0.286	-0.312	-0.223
River Discharge	0.899	0.843	0.756
% Alcaligenes of Total F.A.U.	-0.788	-0.773	-0.580

* At p=0.05 Critical value 0.707

** FAU = Long chain fatty acid utilizer

but does with the total count. There is no inverse relationship at this site in the river between the pH and the FAU ccunt(Tables 37,38 and 39). The presence of long chain fatty acids show a positive significant correlation between with the total count and the pigmented count as is found at the other local sites(Tables 37,38 and 39). These numbers do not correlate with the FAU count. There is a positive correlation between all 3 microbial groups and the organic nitrogen and the total nitrogen present(Table 39). A significant correlation is observed for the 3 microbial types with the river discharge. These latter results are similar to those found at the 156 St. site.

The river water at Vinca bridge appears, in some respects, to be a reflection of a continuing mixture of the water at 156 st. and the sewage effluent. However, due to the effect of the dilution of the sewage effluent by the river water, it can be concluded that the effect of the sewage effluent on the river as shown at Vinca bridge is minimal.

SUMMARY AND CONCLUSION

A direct plating method has been developed for the enumeration of long chain fatty acid utilizing microorganisms. It was observed that almost all colonies growing on the long chain fatty acid containing medium(TYA+FA) could utilize long chain fatty acids. In 1 or 2 instances an isolate was found growing that could not use the substrate. This suggests that the medium is acting in a selective manner since only FAU will grow under these conditions.

The evaluation of the North Saskatchewan river's capacity to utilize long chain fatty acids yielded several conclusions.

1. Long chain fatty acid utilization appears to be secondary with respect to other degradative abilities of the indigenous microflora. One reason for this statement is that the fatty acid concentration does not vary significantly between water samples taken upstream and downstream from Edmonton(Tables 21 and 23). This possibly indicates a sewage effluent dilution effect. The FAU are a significant fraction of the total bacterial population in the North Saskatchewan river which increase 10–100 fold after the river water's passage through Edmonton(Tables 6 and 8). This therefore indicates that there is no selection for a metabolically independant microbial population that can

degrade long chain fatty acids.

2. There is no correlation between the numbers of FAU and fatty acid levels, nitrogen and carbon, again indicating the utilization of the fatty acids to be of a secondary nature.

3. When compared on a volume basis between grossly similar sediments, the Vinca bridge sediment long chain fatty acid content was twice that of the 156 St. sediment(Table 34).

4. Sediments in close proximity to Edmonton(156 st. and Vinca bridge) appear to be quite anaerobic (hydrogen sulphide odor and black in color) when compared to those at the source. There were no facultative anaerobes found at the source, whereas the sediment at both 156 St. and Vinca bridge contained Enterobacteriaceae and Aeromonas sp.

5. The FANU, which are approximately 90% or more of the total bacterial population, show a tendency toward being pigmented(Tables 13,14 and 15).

6. The occurrence of Salmonella sp. in the North Saskatchewan river may be sporadic(Table 19). On 1 occasion an isolation was made at the 156 St. location and the same isolation was made at Vinca bridge a day later. At other times no recoveries were made. The sewage effluent was found to contain Salmonella sp. on 3 out of 8 occasions. The sporadic isolation of Salmonella sp. from the sewage effluent may be due to

the inefficiency of the sampling and enrichment methods.

In conclusion this study shows that the North Saskatchewan river can efficiently degrade or otherwise remove the long chain fatty acids the river receives from the Edmonton Domestic sewage treatment plant. There is, however, a possibility that Vinca bridge sediment is being enriched in fatty acids since the total long chain fatty acid concentration in the sediment is twice that found in the sediment at 156 St. The sediment fatty acids could originate from the Edmonton Domestic sewage treatment plant. This is unlikely as the effluent is discharged at a more or less constant rate throughout the year. This makes it difficult to assess the contribution of long chain fatty acid to the sediment at Vinca bridge by the Edmonton domestic sewage effluent. Another possibility exists in the City's use of the Clover Bar industrial lagoons. These accumulate effluent throughout the winter and are discharged over approximately a 1 month period directly proceeding the breakup. This could have a pulse loading effect on the river allowing more fatty acids to sediment. At the time of the lagoon discharge the river temperature is around 5°C which is low enough to slow degradation tc a point where the fatty acids could sediment unchanged if associated with particulate material.

It is necessary to evaluate the capabilities of the environment for metabolic activities related to the degradation of certain (most) sewage effluent components. There is also no information indicating the relationship between the sediment and the water to the dynamics of nutrient and microbial interchange. To compound this, information is again lacking describing interrelationships between microorganisms and other flora and fauna present in most river systems. In short this study of river microbiology indicates that much work is required for the intelligent use of the river as receiving waters for man's wastes.

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